GENETICS

A periodical record of investigations bearing on heredity and variation

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VOLUME 41 JULY, 1956 NUMBER 4

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PUBLISHED BIMONTHLY AT BALTIMORE, MARYLAND
BY GENETICS, INC.

(Date of issue, August 15, 1956)

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Volume 41 JULY, 1956 Number 4

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $8.00 net a year for complete volumes (January–November). Foreign postage, 50 cents additional. Single copies, $1.50 each, postpaid.

As available, back volumes may be had at $14.00 each and single issues of previous volumes at $2.50 per copy, postpaid. The Business Office will supply information on request as to volumes and numbers available.

Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., Business Office, Mount Royal and Guilford Avenues, Baltimore 2, Maryland.

Remittances should be made payable to GENETICS, INC.

Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

Entered as second-class matter at the postoffice at Baltimore, Md., under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L.&R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

THE THEORY OF THE DIALLEL CROSS[1]

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Received November 4, 1955

RECENTLY there have been extensive discussions of the theory and the analysis of a set of diallel crosses (HULL 1945, 1952; HAYMAN 1954 a, b; JINKS 1954; JINKS and HAYMAN 1953). The purpose of this note is to state very briefly the theory, including arbitrary epistacy and to note some weaknesses of the general method.

We shall take as our starting point a random mating population at equilibrium with genotypic array

$$\prod_{s=1}^{n} \{\sum_{k} p_k^s A_k^s\}^2$$

Here A_k^s is a gene at the sth locus which has gene frequency equal to p_k^s. We shall suppose that inbred lines are obtained from this population *without selection, artificial or natural.* A sample of n lines are obtained and these form the basis for the diallel cross. The parents will be denoted by $P_i (i = 1, 2, \cdots, n)$ and the diallel cross consists of all n parents and the $n(n-1)$ F_1 populations obtained by crossing the n parents two at a time.

We shall suppose that there is:

(1) normal diploid segregation
(2) no difference between reciprocal crosses, that is no maternal effects
(3) arbitrary epistacy
(4) an arbitrary number of alleles at each locus
(5) the parents are homozygous
(6) the phenotypic expression is equal to the sum of a genotypic contribution and an environmental contribution, the latter being associated at random with the genotype.

Actually assumption (2) can be examined from the data. Also assumption (5) is not essential, it being necessary only that the parents have the same coefficient of inbreeding (KEMPTHORNE unpublished).

THE CHARACTERISTICS OF THE ENTRIES OF THE DIALLEL TABLE WITH NO SELECTION WITH ARBITRARY EPISTACY

Let the ith inbred parent be

$$P_i = A_{i_1}^1 A_{i_1}^1 A_{i_2}^2 A_{i_2}^2 \cdots A_{i_n}^n A_{i_n}^n$$

Then the genotype of the F_1 of P_i and P_j is

$$P_i \times P_j = A_{i_1}^1 A_{j_1}^1 A_{i_2}^2 A_{j_2}^2 \cdots A_{i_n}^n A_{j_n}^n$$

[1] Journal Paper No. J-2951 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 890.

Second Printing 1969 / University of Texas Printing Division, Austin

By virtue of the assumption of no selection in the process of obtaining the inbred lines we have

$$P(A^s_{i_s} = A^s_k) = p^s_k ,$$

that is, the probability that the gene at the sth locus of the ith inbred line is the kth allele at that locus is the frequency of this allele which is denoted by p^s_k .

It is clear that the expected value of any F_1 is the mean of the random mating population whose genotypic array is

$$\mu = \prod_{s=1}^{n} \left(\sum_k p^s_k A^s_k\right)^2$$

that is, of the population which would have given rise to the inbred lines by unbreeding without selection.

The expected value of an inbred line is clearly equal to the mean of the population of possible inbred lines and we may denote this by μ_I . We note that in general there will be no relationship between μ and μ_I . The variance of an inbred line similarly will be denoted by σ^2_I which will have no relationship necessarily to the variance in the original population. If there were only additive effects of genes we would have

$$\sigma^2_I = 2\sigma^2_G ,$$

where σ^2_G is the genotypic variance in the original population. This relationship is however extremely sensitive to non-additivity of gene effects.

The variance of an F_1 is equal to the variance in the original population since an F_1 is seen to have the genotypic structure of a random member of the population.

The approximate specification of the entries of the diallel table will be completed by obtaining the covariances of the entries. First we consider the covariance of a parent with an offspring. We see immediately that this is a primitive or intrinsic characteristic of the population in that the gametic array contributed by the parent to the offspring is fixed while the gametic array contributed by the other parent is equal to the gametic array of the original population. Let this covariance be denoted by C.

Finally we have to consider the covariance of the offspring of parents i and j and the offspring of parents i and k say. This is equal to the covariance of

$$\prod_{s=1}^{n} A^s_{i_s} A^s_{j_s}$$

and

$$\prod_{s=1}^{n} A^s_{i_s} A^s_{k_s}$$

and elementary use of the argument of MALÉCOT (1949) or KEMPTHORNE (1954) shows that this is the covariance of two relatives for which φ is unity and φ' is zero. This covariance is then $Cov(P.O)$ in the original population.

We note that the progeny of a pair of parents have the same genotype and hence there is no genotypic variance within cells.

We see therefore that the means, variances and covariances of the entries in the diallel table give us estimates of μ, μ_I, σ^2_G, σ^2_I, C, and $Cov(P.O)$. The covariance is known to be

$$\tfrac{1}{2}\sigma^2_A + \tfrac{1}{4}\sigma^2_{AA} + \tfrac{1}{8}\sigma^2_{AAA} + \text{etc.}$$

in the notation of KEMPTHORNE (1954).

Any analysis of the sum of squares in the diallel table will therefore give mean squares whose expectations are functions of these five parameters. We may derive other covariances from these. For instance the covariance of parental value and the mean of *all* entries in the row of entries for which this is a parent is

$$\frac{1}{n}[\sigma^2_I + (n-1)C].$$

THE PARTICULAR CASE OF NO EPISTACY

In this case let the contributions of genes $A^s_i A^s_j$ to the genotypic value be l^s_{ij} and let

$$\mu^s = \sum_{ij} p^s_i p^s_j l^s_{ij}$$

$$\mu^s_I = \sum_i p^s_i l^s_{ii}$$

Then

$$\mu = \sum_s \mu^s$$

$$\mu_I = \sum_s \mu^s_I$$

$$\sigma^2_G = \sum_s \{\sum_{ij} p^s_i p^s_j (l^s_{ij})^2 - (\mu^s)^2\}$$

$$\sigma^2_I = \sum_s \{\sum_i p^s_i (l^s_{ii})^2 - (\mu^s_I)^2\}$$

$$C = \sum_s \{\sum_i p^s_i l^s_{ii} \alpha^s_i\}$$

where α^s_i is the additive (least squares-wise) effect of gene A^s_i and

$$Cov(P.O) = \sum_s \sum_i p^s_i (\alpha^s_i)^2$$

Here, of course

$$\alpha^s_i = \sum_j p^s_j l^s_{ij} - \mu^s.$$

HAYMAN's formulas (1954b) may be obtained by taking

$$l^s_{11} = d_s \qquad p^s_1 = w_s$$

$$l^s_{12} = h_s \qquad p^s_2 = 1 - w_s$$

$$l^s_{22} = -d_s$$

We may further particularize the results of the previous section in the case where there are two equally frequent alleles at each locus. This would arise if the inbred

lines were obtained by inbreeding an F_2 population arising from two inbred lines. We will translate to MATHER's notation at this point in the following way:

$$l_{11}^s = d_s$$

$$l_{12}^s = h_s$$

$$l_{22}^s = -d_s$$

We would then have

$$\mu^s = \tfrac{1}{2}h_s$$

$$\mu_I^s = 0$$

$$\mu = \tfrac{1}{2}\sum h_s$$

$$\mu_I = 0$$

$$\sigma_G^2 = \tfrac{1}{2}D + \tfrac{1}{4}H \text{ where } D = \sum d_s^2 , H = \sum h_s^2$$

Also

$$\alpha_1^s = -\alpha_2^s = \tfrac{1}{2}d_s$$

so that

$$C = \tfrac{1}{2}D,$$

and $Cov(P.O) = \tfrac{1}{4}D$.

THE ANALYSIS OF VARIANCE OF THE DIALLEL TABLE

An analysis of variance of the entries in the diallel table has been given by HAYMAN (1954a) following YATES (1947). We shall use the notation

y_{ij} = phenotypic value of individual with P_i as male parent and P_j as female parent.

and

$$Y_{i\cdot} = \sum_j y_{ij}, Y_{\cdot j} = \sum_i y_{ij}, Y_{\cdot\cdot} = \sum_{ij} y_{ij} .$$

YATES's analysis of variance is given in table 1. In this table

$$L = \sum_r \frac{(Y_{r\cdot} + Y_{\cdot r})^2}{2n} - 2\frac{Y_{\cdot\cdot}^2}{n^2}$$

$$C = \sum_{rs} \frac{(y_{rs} + y_{sr})^2}{4} - \sum_r \frac{(Y_{r\cdot} + Y_{\cdot r})^2}{2n} + \frac{Y_{\cdot\cdot}^2}{n^2}$$

$$M = \sum_r \frac{(Y_{r\cdot} - Y_{\cdot r})^2}{2n}$$

$$R = \sum_{rs} \frac{(Y_{rs} - Y_{sr})^2}{4} - \sum_r \frac{(Y_{r\cdot} - Y_{\cdot r})^2}{2n}.$$

We shall now obtain the expectations of the sum of squares under the assumptions listed above. We do this in steps.

TABLE 1

An analysis of variance of the diallel table

Source	d.f.	Sum of Squares	Mean Square
Lines	$n-1$	L	L^*
Reciprocal sums after lines	$\frac{1}{2}n(n-1)$	C	C^*
Average maternal effects	$n-1$	M	M^*
Additional reciprocal differences	$\frac{1}{2}(n-1)(n-2)$	R	R^*

(1) $\frac{2Y_{..}^2}{n^2}$ has expectation equal to

$$2[\mu_I + (n-1)\mu]^2 + \frac{2}{n}\sigma_I^2 + 4\frac{(n-1)}{n}\sigma_G^2 + 8\frac{(n-1)}{n}C + 8\frac{(n-1)(n-2)}{n}\,Cov(P.O)$$

(2) $\sum_r \frac{(Y_{r.} + Y_{.r})^2}{2n}$ has expectation equal to

$$2[\mu_I + (n-1)\mu]^2 + 2\sigma_I^2 + 2(n-1)\sigma_G^2 + 4(n-1)C + 2(n-1)(n-2)\,Cov(P.O)$$

(3) $\sum_{rs} \frac{(y_{rs} + y_{sr})^2}{4}$ has expectation equal to

$$n\mu_I^2 + n(n-1)\mu^2 + n\sigma_I^2 + n(n-1)\sigma_G^2$$

(4) $\sum_r \frac{(Y_{r.} - Y_{.r})^2}{2n}$ has expectation equal to zero under the assumption of equality of reciprocal crosses.

(5) $\sum_{rs} \frac{(y_{rs} - y_{sr})^2}{4}$ has expectation equal to zero under the assumption of equality of reciprocal crosses.

and finally as a check

(6) $\sum_{rs} y_{rs}^2$ has expectation equal to

$$n\mu_I^2 + n(n-1)\mu^2 + n\sigma_I^2 + n(n-1)\sigma_G^2.$$

Of course each sum of squares will also contain its degrees of freedom times σ^2 where σ^2 is the environmental variance. We therefore see that

$$E(L^*) = \sigma^2 + \frac{2}{n}\sigma_I^2 + 2\frac{(n-2)}{n}\sigma_G^2 + 4\frac{(n-2)}{n}C + 2\frac{(n-2)(n-4)}{n}\,Cov(P.O)$$

$$E(C^*) = \sigma^2 + \frac{2}{n}(\mu_I - \mu)^2 + 2\frac{(n-1)}{n^2}\sigma_I^2 + 2\frac{(n^2-2n+2)}{n^2}\sigma_G^2 - 8\frac{(n-1)}{n^2}C - 4\frac{(n-2)^2}{n^2}\,Cov(P.O)$$

$$E(M^*) = \sigma^2$$

and

$$E(R^*) = \sigma^2.$$

These general results need little comment except to state that this analysis of variance is useless under the present assumptions for estimating intrinsic population characteristics.

It is however of interest to record what happens if there is no epistacy and no dominance. We then have

$$\mu_I = \mu \qquad \sigma_G^2 = 2\,Cov(P.O), \qquad \sigma_I^2 = 2\sigma_G^2 \qquad C = \sigma_G^2$$

and

$$\begin{aligned} E(L^*) &= \sigma^2 + n\sigma_G^2 \\ E(C^*) &= \sigma^2 \\ E(M^*) &= \sigma^2 \\ E(R^*) &= \sigma^2 \end{aligned}$$

so that L^* may be compared with C^* to estimate σ_G^2.

In the case when there is no epistacy it is instructive to write $E(C^*)$ as follows:

$$E(C^*) = \sigma^2 + \frac{2}{n}(\mu_I - \mu)^2 + 2\frac{(n-1)}{n^2}\{\sigma_I^2 + 2\sigma_G^2 - 4C\} + 2\frac{2(n-2)^2}{n^2}\{\sigma_G^2 - 2\,Cov(P.O)\}.$$

Now let us write for any locus

$$y_{ij} = \mu + \alpha_i + \alpha_j + d_{ij}$$

so that d_{ij} is the least squares dominance deviation. Then

$$\begin{aligned} &\sigma_I^2 + 2\sigma_G^2 - 4C \\ &= \sum_i p_i(y_{ii}^2 - (\sum_i p_i y_{ii})^2 + 2\sum_{ij} p_i p_j y_{ij}^2 - 2\mu^2 - 4\sum p_i y_{ii}\alpha_i \\ &= \sum_i p_i(2\alpha_i + d_{ii})^2 - [\sum_i p_i(2\alpha_i + d_{ii})]^2 + 4\sum p_i\alpha_i^2 + 2\sum_{ij} p_i p_j d_{ij}^2 \\ &\qquad - 4\sum_i p_i(2\alpha_i + d_{ii})\alpha_i \\ &= \sum_i p_i\, d_{ii}^2 - (\sum_i p_i d_{ii})^2 + 2\sum_{ij} p_i p_j\, d_{ij}^2 \\ &= \sum_i p_i\{d_{ii} - \sum_k p_k\, d_{kk}\}^2 + 2\sum_{ij} p_i d_{ij}^2 . \end{aligned}$$

Hence $\sigma_I^2 + 2\sigma_G^2 - 4C$ is a positive definite quadratic form in the dominance deviations. Similarly $\sigma_G^2 - 2\,Cov(P.O)$ is the dominance variance and is $\sum_{ij} p_i p_j\, d_{ij}^2$. Also $(\mu_I - \mu) = \sum_i p_i\, d_{ii} - \sum_{ij} p_i p_j\, d_{ij}$. We see therefore that in the case of no epistacy the genotypic part of the expectation of C^* is a positive definite form in the

dominance deviations only and hence is zero only if the dominance deviations are all zero. This result is equivalent to HAYMAN's (1954a) result and indicates that C^* may be compared with M^* and R^* separately or pooled to test for dominance if there is no epistacy.

It is of interest to examine the partition of the sum of squares C given by HAYMAN (1954). He breaks C into three parts which we denote here as follows:

$$C_1 = (Y_{..} - n\sum_i y_{ii})^2/n^2(n-1) \text{ with 1 degree of freedom}$$

$$C_2 = \sum_r \{Y_{r.} + Y_{.r} - ny_{rr} - 2Y_{..} + \sum_i y_{ii}\}^2/n(n-2)$$

or

$$\sum_r \{Y_{r.} + Y_{.r} - ny_{rr}\}^2/n(n-2) - \{2Y_{..} - n\sum_i y_{ii}\}^2/n^2(n-2)$$

with $(n-1)$ degrees of freedom and

$C_3 = C - C_1 - C_2$ with $\frac{1}{2}n(n-3)$ degrees of freedom.

We find the expectations of the first two mean squares to be as follows:

$$E(C_1^*) = \sigma^2 + (n-1)(\mu_I - \mu)^2 + \frac{(n-1)}{2}\sigma_I^2 + \frac{2}{n}\sigma_G^2 - 4\frac{(n-1)}{n}C + 4\frac{(n-2)}{n}Cov(P.O)$$

$$E(C_2^*) = \sigma^2 + \frac{(n-2)}{n}\sigma_I^2 + \frac{4}{n}\sigma_G^2 - 4\frac{(n-2)}{n}C + 4\frac{(n-4)}{n}Cov(P.O)$$

and the expectation of C_3^* can be obtained by subtraction. It does not appear that these mean squares can have any general utility, though in very special circumstances as indicated by HAYMAN (1954a) they do have some value.

DISCUSSION

We see from the above that only under very special circumstances, in particular the absence of epistacy, can the diallel table give us any information about the intrinsic properties of the original population. This is true because under the assumptions listed at the beginning the expectations of the mean squares given by YATES (1947) and used by HAYMAN (1954a) are linear function of the following quantities:

(1) $(\mu_I - \mu)^2$, that is, the square of the difference of the original population mean and the mean of all possible homozygous lines that can be derived without selection from that population.
(2) σ_G^2, which is the total genotypic variance of the original population.
(3) σ_I^2, which is the variance in the population of possible inbred lines.
(4) C, which is the covariance of inbred lines and the progeny of the inbred lines and the original population.

and

(5) $Cov(P.O)$, which is the covariance of parent and offspring in the original population.

It is apparent however from the development given herein that the six quantities μ, μ_I, σ_I^2, σ_G^2, C and $Cov(P.O)$ can be estimated though not by an orthogonal analysis of variance. These quantities appear to have definite genetic value and it appears that the analysis of the diallel table should be directed to their estimation. The estimation procedure will not be given here.

The assumptions stated by HAYMAN (1954b) in his description of the situation are our assumptions (1), (2), (5) and (6) (essentially, with the minor modification that the environmental variance for parents and F_1's may be different) together with the following (using his numbering)

(iii) independent action of non-allelic genes

(iv) no multiple allelism

(vi) genes independently distributed between the parents.

We have removed assumptions (iii) and (iv) and the origin of the parents that we have assumed would have (vi) as a result. The assumption (iv) would be true if our original population were an F_2 of two homozygous lines.

HAYMAN's assumption (vi) is the particular one which should be singled out for discussion. In the situation we have postulated the assumption will be valid but the crucial question is whether the assumption can be true with a set of lines of unknown origin which appears to be the case with those used by JINKS (1954).

It is worth noting that if we use an arbitrary set of n parents and do not make HAYMAN's assumption (vi) but grant all his other assumptions the situation is hopeless. In fact under these circumstances there will be 2^{n-1} loci groups (ANDERSON and KEMPTHORNE 1954) to each of which will be attached an additive (or direct) effect of one allele over the other and a dominance effect. The number of parameters of this type operating in the situation is then $2^n - 1$, including the basal level and there are only $\frac{1}{2}n(n+1)$ different genotypic values to be explained in terms of these parameters.

The diallel cross must be interpreted in terms of some population which has given rise to the homozygous parents by inbreeding. If such a population does not exist then the whole analysis envisaged above is likely to lead nowhere. From quite another viewpoint one may question the value of estimating additive variance, dominance variance and so on, however they may be defined, unless the estimated quantities are measures of the characteristics of a definite population. The estimates can have relevance only to a particular population and will not be constant from population to population. If these considerations be accepted the scientific or logical value of removing some of the parents from the diallel table and considering the remainder (JINKS 1954) appears to be nil. Even if the remainder should exhibit no epistacy by particular tests and one then goes ahead and estimates additive variance and dominance variance, the real question remains as to what population the estimates are applicable. Even if there is a case for regarding the whole set of parents as a random sample of the lines which could be obtained by inbreeding a population, there does not appear to be any logical basis for regarding a subset of the parents, chosen on the basis of their performance, as being a random sample derived from another population.

CONCLUSION

A general approach to the genetical theory and analysis of the diallel table is presented. The assumptions of two alleles at each locus and no epistacy are removed. It is seen however that the analysis of variance used in the past for diallel cross is of little use unless epistacy can be ignored. The general theory given herein suggests that quantities of genetic interest can be estimated, but not by previously used methods.

It is pointed out that selection of material within the diallel table and analysis of the remainder has questionable logical value.

ACKNOWLEDGMENT

It is a pleasure for the author to acknowledge helpful discussions on the problem with R. E. COMSTOCK and J. B. GRIFFING.

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PARENT-OFFSPRING AND FULL SIB CORRELATIONS UNDER A PARENT-OFFSPRING MATING SYSTEM[1]

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Received February 25, 1956

USING the method of path coefficients, WRIGHT (1921) derived correlations among relatives in inbred populations for the special case of no dominance or epistasis. FISHER (1947) described the use of generation matrix methodology in the theory of inbreeding, some of this methodology being due to earlier writers. FISHER's concern was with mating types, where the matings $AA \times AA$ and $aa \times aa$, for instance, are of the same type.

More recently, KEMPTHORNE (1955) pointed out two distinct types of problems involving correlations between relatives in inbred populations. Assume that a random mating population is inbred according to a regular system of inbreeding. In generation n we have the population

$$(p^2 + F_n pq)AA + 2(1 - F_n)pqAa + (q^2 + F_n pq)aa$$

where F_n is the coefficient of inbreeding attained at generation n. The first problem is represented by the following: (1) what is the correlation between full sibs in generation n and (2) what is the correlation between an individual of that generation and his offspring in generation $n + 1$? For the second problem, suppose that the above population is in equilibrium, then what is the correlation between relatives in this population? This same paper dealt with the first problem, using generation matrix methodology and assuming two alleles per locus, for a full sib inbreeding system. Kinds of matings were employed rather than types; i.e., $AA \times AA$ is a different kind of mating from $aa \times aa$.

The objective in the present paper was to obtain parent-offspring and full sib correlations in the nth generation under a parent-offspring mating system. This necessitates obtaining the frequencies of the various kinds of matings in the nth generation as a function of the frequencies in the zero generation.

PARENT-OFFSPRING MATING SYSTEM

The offspring of a mating is always mated to the younger parent. Considering a single locus with two alleles per locus there are nine kinds of matings, which are shown in table 1. These are arbitrarily numbered from one to nine. Mating kind frequencies will be indicated as $f_1, f_2, \cdots, f_9$ with superscripts in parenthesis indicating the appropriate generation. Thus $f_1^{(3)}$ is the frequency of the first kind of mating in the third generation.

[1] Journal Paper No. J-2976 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project number 1285.

TABLE 1

Kinds of matings

Kind of mating: Younger parent		Older parent	Designation	Frequency in generation zero	Proportions of kinds of matings resulting from mating of a given kind: Kind of mating 1	2	3	4	5	6	7	8	9
Y		Z											
AA	×	AA	1	$f_1^{(0)}$	1								
AA	×	Aa	2	$f_2^{(0)}$	$\frac{1}{2}$			$\frac{1}{2}$					
AA	×	aa	3	$f_3^{(0)}$				1					
Aa	×	AA	4	$f_4^{(0)}$		$\frac{1}{2}$			$\frac{1}{2}$				
Aa	×	Aa	5	$f_5^{(0)}$		$\frac{1}{4}$			$\frac{1}{2}$			$\frac{1}{4}$	
Aa	×	aa	6	$f_6^{(0)}$					$\frac{1}{2}$			$\frac{1}{2}$	
aa	×	AA	7	$f_7^{(0)}$									
aa	×	Aa	8	$f_8^{(0)}$						1			$\frac{1}{2}$
aa	×	aa	9	$f_9^{(0)}$						$\frac{1}{2}$			1

KIND OF MATING FREQUENCIES IN THE F_n GENERATION

The kind of mating frequencies in the first generation are linear functions of the kind of mating frequencies in the zero generation. These linear functions are obtained in the following manner.[2] Mating kind *one* leads to mating kind *one*. The offspring from mating kind *two* ($AA \times Aa$) are $\frac{1}{2}AA$ and $\frac{1}{2}Aa$. These are mated to the younger parent which in this case is AA; and which becomes the older parent in reference to the new generation. Hence a mating of kind *two* leads to matings of which one half are of kind *one* and one half are of kind *four*. In the right half of table 1 are shown the proportions of the various kinds of matings which result from the kind of mating indicated in the left hand column. The frequency of mating i in the first generation can be obtained from table 1 as the sum of products of corresponding elements in the "frequency in generation zero" column and the "ith kind of mating" column to the right.

If we let $(f^{(n)})' = (f_1^{(n)} f_2^{(n)} \cdots f_9^{(n)})$ be a column matrix, then kind of mating frequencies in the first generation relate to those in the zero generation by the equation $f^{(1)} = Af^{(0)}$ where A is a 9×9 matrix. Using generation matrix methodology, it can be shown that $f^{(n)} = C^{-1}\Lambda^n C f^{(0)}$ where Λ is a diagonal matrix whose elements along the diagonal are the latent roots of the A matrix. In the present case these roots are $1, 1, 0, 0, 0, -\frac{1}{2}, \frac{1}{2}, \epsilon$ and ϵ' where $\epsilon = \frac{1}{4}(1 + \sqrt{5})$ and $\epsilon' = \frac{1}{4}(1 - \sqrt{5})$. The C matrix is any matrix satisfying the equation $CAC^{-1} = \Lambda$. Assuming that the genotypic array in the zero generation is $p^2(AA) + 2pq(Aa)$ and $q^2(aa)$, the $f^{(n)}$ matrix is

[2] A more detailed derivation is available from the author in mimeographed form.

$$f^{(n)} = \begin{pmatrix} p + pq(1-2p)\,(\tfrac{1}{2})^n - \tfrac{1}{5}pq(3S_n + 8S_{n+1}) + p^2q^20^n \\ \quad - pq(1-2p)\,(\tfrac{1}{2})^n + \tfrac{1}{5}pq(2S_n + 2S_{n+1}) - 2p^2q^20^n \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad p^2q^20^n \\ \quad - pq(1-2p)\,(\tfrac{1}{2})^n - \tfrac{1}{5}pq(S_n - 4S_{n+1}) \;+ pq(p^2+q^2)0^n \\ \qquad\qquad\qquad\qquad\qquad\qquad \tfrac{1}{5}pq(4S_n + 4S_{n+1}) - 2pq(p^2+q^2)0^n \\ \qquad pq(1-2p)\,(\tfrac{1}{2})^n - \tfrac{1}{5}pq(S_n - 4S_{n+1}) \;+ pq(p^2+q^2)0^n \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad p^2q^20^n \\ \qquad pq(1-2p)\,(\tfrac{1}{2})^n + \tfrac{1}{5}pq(2S_n + 2S_{n+1}) - 2p^2q^20^n \\ q - pq(1-2p)\,(\tfrac{1}{2})^n - \tfrac{1}{5}pq(3S_n + 8S_{n+1}) + p^2q^20^n \end{pmatrix}$$

In the above matrix $S_n = \epsilon^n + (\epsilon')^n$ and $S_{n+1} = \epsilon^{n+1} + (\epsilon')^{n+1}$.

PARENT OFFSPRING COVARIANCE

The values of the genotypes AA, Aa and aa will be represented as y, $\frac{1}{2}(y - x)$ and 0. On this scale the degree of dominance is $-x/y$. The parent offspring covariance is

$$Cov(P_{n-1}, O_n) = f_1^{(n)}(AA)(AA) + f_2^{(n)}(AA)(Aa) + \cdots f_9^{(n)}(aa)(aa) - \mu_n\mu_{n-1}$$

where

$$\mu_n = (AA)(f_1^{(n)} + f_2^{(n)} + f_3^{(n)}) + (Aa)(f_4^{(n)} + f_5^{(n)} + f_6^{(n)}) + (aa)(f_7^{(n)} + f_8^{(n)} + f_9^{(n)})$$

and

$$\mu_{n-1} = (AA)(f_1^{(n)} + f_4^{(n)} + f_7^{(n)}) + (Aa)(f_2^{(n)} + f_5^{(n)} + f_8^{(n)}) + (aa)(f_3^{(n)} + f_6^{(n)} + f_9^{(n)})$$

In the above formula genotypic values are to be inserted for genotypes. After such substitution, collection of terms in y^2, xy and x^2, and substitution of

$$\tfrac{1}{2} - \tfrac{3}{2}F_{n-1} + F_n$$

for S_n (see KEMPTHORNE 1955) and $\frac{3}{4} + \frac{1}{4}F_{n-1} - F_n$ for S_{n+1}, we have $Cov(P_{n-1}, O_n)$ in terms of WRIGHT's coefficient of inbreeding F. Thus

$$Cov(P_{n-1}, O_n) = pq(\tfrac{1}{4} + \tfrac{1}{4}F_{n-1} + \tfrac{1}{2}F_n)y^2 + pq(1-2p)\,[(\tfrac{1}{2})^n - 1 + \tfrac{1}{2}F_{n-1} + \tfrac{1}{2}F_n]xy + \tfrac{1}{4}pq(1 - F_{n-1})\,[(1-2p)^2 + 4pqF_n]x^2 \qquad n \geq 1$$

The variance in the nth generation is

$$\sigma_Y^2 = (AA)^2\,(f_1^{(n)} + f_2^{(n)} + f_3^{(n)}) + (Aa)^2\,(f_4^{(n)} + f_5^{(n)} + f_6^{(n)}) + (aa)^2(f_7^{(n)} + f_8^{(n)} + f_9^{(n)}) - \mu_n^2$$

or

$$\sigma_Y^2 = \tfrac{1}{2}pq(1 + F_n)y^2 - pq(1-2p)\,(1 - F_n)xy + \tfrac{1}{2}pq[1 - F_n - 2pq(1 - F_n)^2]x^2$$

The variance in the $n-1$ generation, σ_Z^2, can be obtained from σ_Y^2 by substitution

of $(n-1)$ for n. The genotypic correlation of parents in the $n-1$ generation with their offspring in the nth generation is

$$\frac{Cov(P_{n-1}, O_n)}{\sigma_Y \sigma_Z}$$

FULL SIB COVARIANCE

Each kind of mating in the nth generation gives rise to a progeny in the $(n+1)$ generation; the frequency of each progeny being the same as the frequency of each kind of mating in the nth generation. The covariance of full sibs in the $(n+1)$th generation is the same as the variance among progeny means in the $(n+1)$th generation.

Thus

$$Cov(\textit{Full sibs in generation } n+1) = (AA \times AA)^2 f_1^{(n)} + (AA \times Aa)^2 f_2^{(n)} + \cdots (aa \times aa)^2 f_9^{(n)} - \mu_{n+1}^2$$

where progeny means are to be inserted for the indicated matings.

After simplifying the above equation and substitution of $n-1$ for n we have

$$Cov(\textit{Full sib in generation } n) = pq(\tfrac{1}{8} + \tfrac{1}{8}0^{n-1} - \tfrac{1}{8}F_{n-1} + F_n)y^2 + pq(1-2p)\,[(\tfrac{1}{2})^n - (1-F_n)]xy + pq[\tfrac{1}{4}pq0^{n-1} + \tfrac{1}{4}(1-F_n) - pq(1-F_n)^2]x^2$$

The genotypic correlation among full sibs is

$$\frac{Cov(\textit{Full sib in generation } n)}{\sigma_Y^2}$$

ADDITIVE GENETIC VARIANCE

The additive genetic variance of the nth generation in the case of 2 alleles is the sum of squares attributable to regression of genotypic value upon the number of A genes of the genotype. The variance is

$$\sigma_A^2 = \frac{[Cov\ (\textit{Genotypic Value, Number of A genes})]^2}{\textit{Variance of number of A genes}}$$

In the nth generation the covariance of the numerator is

$$[f_1^{(n)} + f_2^{(n)} + f_3^{(n)}](2)y + [f_4^{(n)} + f_5^{(n)} + f_6^{(n)}](1)\left(\frac{y-x}{2}\right) + [f_7^{(n)} + f_8^{(n)} + f_9^{(n)}](0)\,(0) - \{[f_1^{(n)} + f_2^{(n)} + f_3^{(n)}](2) + [f_4^{(n)} + f_5^{(n)} + f_6^{(n)}](1) + [f_7^{(n)} + f_8^{(n)} + f_9^{(n)}](0)\}\mu_n$$

and the denominator is

$$[f_1^{(n)} + f_2^{(n)} + f_3^{(n)}]2^2 + [f_4^{(n)} + f_5^{(n)} + f_6^{(n)}]1^2 + [f_7^{(n)} + f_8^{(n)} + f_9^{(n)}]0^2 - \{[f_1^{(n)} + f_2^{(n)} + f_3^{(n)}](2) + [f_4^{(n)} + f_5^{(n)} + f_6^{(n)}](1) + [f_7^{(n)} + f_8^{(n)} + f_9^{(n)}]0\}^2$$

Hence

$$\sigma_A^2 = \tfrac{1}{2}p(1-p)\left\{(1+F_n)y^2 + 2(2p-1)(1-F_n)xy + (1-2p)^2\frac{(1-F_n)^2}{1+F_n}x^2\right\}$$

In the random mating population (generation 0 or 1) the additive genetic variance is

$$\sigma_A^2 = \tfrac{1}{2}p(1-p)\{y + (2p-1)x\}^2$$

PARENT OFFSPRING AND FULL SIB CORRELATIONS

The relationship between these correlations and generation (n) are shown in graphs 1a (parent offspring) and 1b (full sib) for selected degrees of dominance and gene frequencies. Degree of dominance equals $-\dfrac{x}{y} = a$, say.

In the case of no dominance these correlations, which in the first generation are one half, rise to *one* as $n \to \infty$, with the parent offspring correlations being somewhat higher, particularly in the early generations, than the full sib correlations. When dominance is present these correlations are decreased as the frequency of the A allele increases, particularly in the early generations, which may have correlations considerably less than one half. This decrease is not as drastic for the full sib correlation as for the parent offspring correlation. When $y = 0$, degree of dominance is infinite and the parent offspring correlation approaches $\sqrt{-E'} = .5559$ as $n \to \infty$ and the full sib correlation approaches $\frac{1}{2}$ as $n \to \infty$.

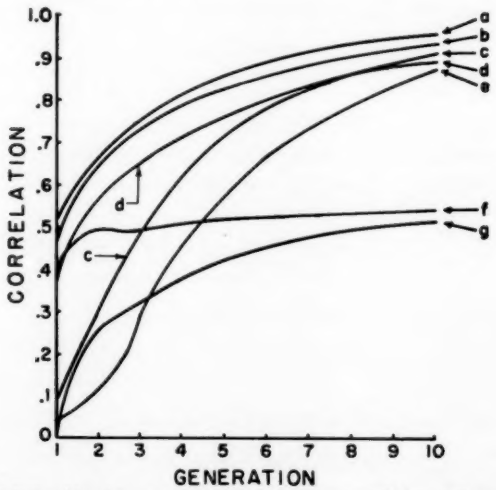

FIGURE 1a.—Relationships between the parent offspring correlation and generation. Case (a) $a = 0$, all values of p; (b) $a = 1$, $p = .1$; (c) $a = 1$, $p = .9$; (d) $a = 1.5$, $p = .3$; (e) $a = 1.5$, $p = .9$; (f) $a = \infty$, $p = .1$ or .9, (g) $a = \infty$, $p = .5$.

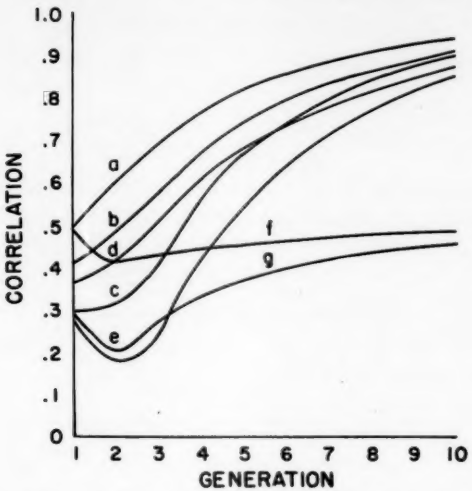

FIGURE 1b.—Relationships between the full sib correlation and generation. Case (a) $a = 0$, all values of p; (b) $a = 1$, $p = .5$; (c) $a = 1$, $p = .9$; (d) $a = 1.5$, $p = .5$; (e) $a = 1.5$, $p = .9$; (f) $a = \infty$, $p = .1$ or .9; (g) $a = \infty$, $p = .5$.

ESTIMATION OF ADDITIVE GENETIC VARIANCE OF ORIGINAL RANDOM MATING POPULATION

Parent Offspring covariance procedure

The additive genetic variance in the original random breeding population on the assumption of no dominance is $\frac{1}{2}p(1 - p)y^2$ and the covariance of parent in the $(n - 1)$ generation and offspring in the nth generation is

$$\tfrac{1}{4}p(1 - p)\,(1 + F_{n-1} + 2F_n)y^2$$

which suggests estimating the additive genetic variance in the random mating population by

$$\widehat{\sigma^2_{A(PO)}} = \frac{2\widehat{Cov}(P_{n-1},O_n)}{1 + F_{n-1} + 2F_n}$$

This procedure is, of course, unbiased when there is no dominance. The bias for various gene frequencies and degrees of dominance is graphed against generation in figure 2a. When gene frequency is one half, the bias may be of the order of 10% in early generations for the case of complete dominance. The bias increases as the degree of dominance increases and as p deviates from one half. For example in the case of complete dominance and a gene frequency of .7, the bias using the covariance of parents in the $n - 1 = 4$ generation and offspring in $n = 5$ generation is 122%.

Full sib covariance procedure

Assuming no dominance, an unbiased estimate of σ^2_A in the original random mating population is

$$\widehat{\sigma^2_{A(F.S)}} = \frac{2\widehat{Cov}\ (F.S.\ in\ generation\ n)}{[\frac{1}{2}(1 + 0^{n-1}) - \frac{1}{2}F_{n-1} + 4F_n]}$$

The bias from this procedure is illustrated in figure 2b. The curves are similar to those of figure 1b in that the percentage bias approaches the same limit as n increases. They differ in that the bias need not be zero in the first generation and in general $\widehat{\sigma^2_{A(F.S)}}$ is larger than $\sigma^2_{A(P.O)}$ for corresponding cases.

Total genotypic variance procedure

A third unbiased estimate, assuming no dominance, of the additive genetic variance in the random mating population is

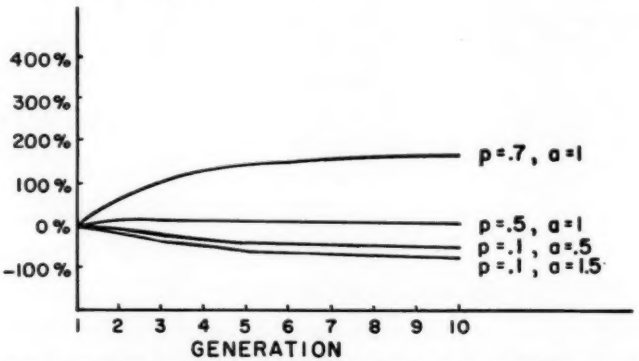

FIGURE 2a.—Percentage bias in each generation of the estimate of the additive genetic variance of the random mating population by $\widehat{\sigma^2_{A(P.O.)}}$.

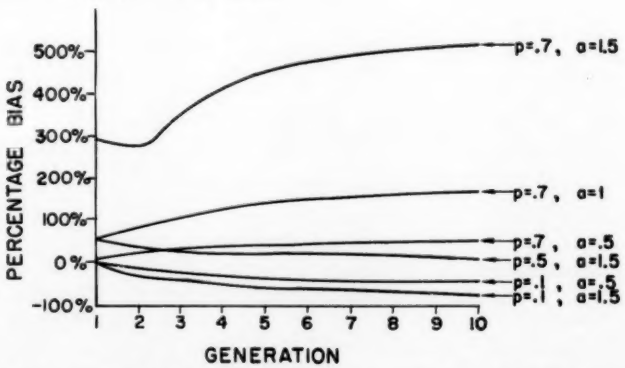

FIGURE 2b.—Percentage bias in each generation of the estimate of the additive genetic variance of the random mating population by $\widehat{\sigma^2_{A(F.S)}}$.

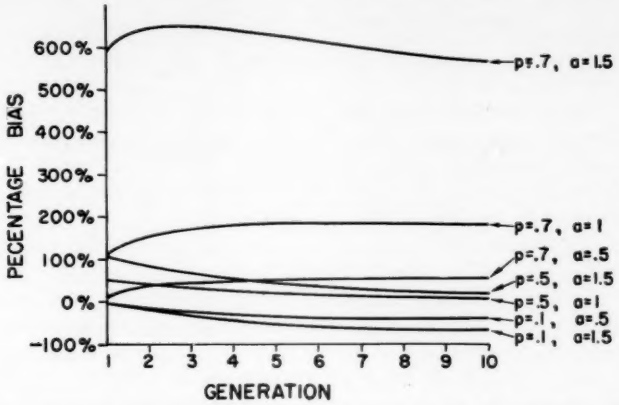

Figure 2c.—Percentage bias in each generation of the estimate of the additive genetic variance of the random mating population by $\widehat{\sigma^2}_{A(T.G.V)}$.

$$\widehat{\sigma^2}_{A(T.G.V)} = \frac{\widehat{\sigma^2_Y}}{1 + F_n}$$

and the bias is graphed in figure 2c against generation. As n increases the bias approaches the same limit as before. In early generations the positive bias is larger (or the negative smaller) than the corresponding cases for the parent offspring and full sib procedures.

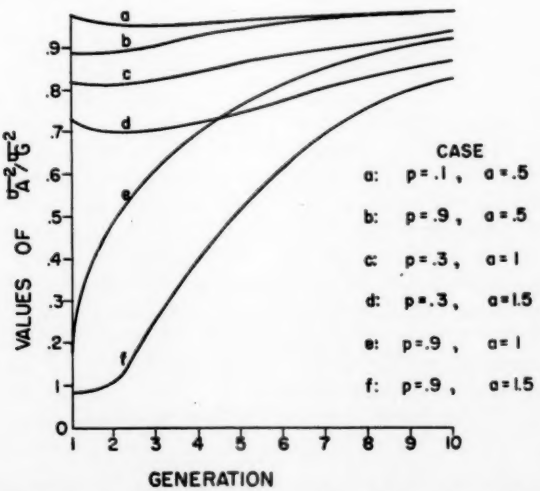

FIGURE 3.—The additive genetic fraction of the total genotypic variance in the nth generation; the additive genetic variance being defined in the nth generation.

PARTITION OF THE TOTAL GENOTYPIC VARIANCE

The proportion of the additive genetic variance, when this variance is defined in the F_n generation, of the total genotypic variance is graphed against generation in figure 3. As n increases the additive fraction tends to constitute, of course, the whole of the genotypic variance. In early generations for the higher gene frequencies and degrees of dominance, this fraction may be quite low. For example in the case of complete dominance and $p = .9$, the additive genetic variance is only (60%) of the total genotypic variance in the 3rd generation.

SUMMARY

Parent offspring and full sib correlations in the nth generation are derived for a parent offspring mating system using generation matrix techniques. These correlations are reduced when dominance is present and in early generations may be considerably less than one half. Biases in the estimate of additive genetic variance of the random breeding population by (a) a parent offspring procedure, (b) a full sib procedure and (c) a total genotypic variance procedure are small when gene frequency is close to one half for partial or complete dominance. When dominance is present, considerable positive or negative bias may result, depending on whether gene frequency is larger or smaller than one half, the bias becoming larger as the degree of dominance increases.

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COMPETITION BETWEEN MUTANTS IN EXPERIMENTAL POPULATIONS OF DROSOPHILA MELANOGASTER[1]

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Received December 12, 1955

ONE of the truisms of modern biology is that organisms are adapted to their physical and biological environments. No evolutionary change is possible which does not at the same time leave the organism adapted to its environment. Although the forces of natural selection, mutation, migration and random genetic drift may interact to produce evolutionary changes, natural selection has, since the time of Darwin, assumed a dominant role because it is the mechanism of adaptation. Natural selection and hence adaptation hinge on differential reproduction, a greater proportion of the better adapted types surviving to reproduce. Despite the fundamental role played by adaptation in relation to a great variety of biological problems, and despite the widespread interest in adaptive phenomena, a feeling exists that much still remains to be learned about adaptation. One of the major changes in SIMPSON's "The Major Features of Evolution" (1953) as compared to his "Tempo and Mode in Evolution" (1944) was the inclusion of an extensive discussion of adaptation. And in his review of this latest book by SIMPSON, STEBBINS (1954) writes, in relation to the section on adaptation, "Perhaps the greatest gap in modern evolutionary knowledge is our paucity of precise information on just how and why certain characters are selected under a given set of environmental conditions." A similar opinion was expressed by WADDINGTON (1953) who further felt that although the mathematical theories of population genetics have had considerable impact on evolutionary thought, the validity and usefulness of some of the concepts have yet to be adequately demonstrated. The current discussion of the significance to evolution of random genetic drift is a case in point.

The experiments to be reported are attempts to explore some of the problems related to the adaptive value of particular genes. It has recently been suggested (DOBZHANSKY and WALLACE 1954) that the study of adaptive differences should involve polygenically controlled traits rather than single factor differences. Since it is impossible to set up separate classes of major and minor genes, and since so far as is known the same principles govern the behavior of genes of all kinds, an approach which involves genes causing major phenotypic and adaptive differences may help to extend the theories and point the way to the more complex studies involving polygenic traits. Our knowledge of the adaptive values of even the simply inherited, clear-cut traits is still rather sketchy. On the list of "frankly deleterious variants" cited by DOBZHANSKY and WALLACE were Huntington's chorea and sickle cell anemia. However, Huntington's chorea has been shown to raise the reproductive level of carriers above that of their normal sibs (REED and PALM 1951), and ALLISON

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and University of Minnesota, NR164–111.

(1954) has recently presented evidence showing that persons with sickle cell trait (the heterozygotes for the gene producing sickle cell anemia) are less susceptible to subtertian malaria. Hence, the evaluation of the adaptive value, even of a gene which seemingly must be deleterious, needs to rest on a careful appraisal of all of its effects in the population. At the present time, the most rapid progress toward an increased understanding of the concept of adaptive value seems possible from work with genes having well-defined effects and clear-cut inheritance.

METHODS

In order to determine the relative adaptive value of a pair of alleles, flies carrying these genes were placed in a "population bottles" devised by REED and REED (1948) and LUDWIN (1951). A population unit consists of two half-pint milk bottles joined together by a 3-inch section of automobile radiator hose. A $\frac{3}{4}$-inch hole for ventilation is cut into the hose and plugged with cotton. The food is poured into bottles as they lie on their sides and hardens in a "slant". A fresh slant of food is used to replace each bottle alternately as its food is exhausted. In this way, a population can be maintained for an indefinite period of time with a minimum amount of handling or disturbance. A major advantage of the population bottles over population cages (e.g. WRIGHT and DOBZHANSKY 1946) is that the units are small and easily handled so that a number of replicas of each experiment can be run.

The food used was a standard corn meal, corn syrup, brewer's yeast, agar medium containing Moldex, to which were added a few drops of live yeast suspension. The populations were maintained in a constantly lighted room held at 21 $\pm$ 1°C and 50% relative humidity.

The populations were sampled by removing the cotton plug from the hose connection and placing a vial over the hole, with the vial held horizontally toward a fluorescent light. The samples were taken a few days after a new slant had been added so that the females had had an opportunity to lay their eggs on the fresh food. The flies usually walked slowly rather than flew into the vial so that position with relation to the opening rather than activity seemed to govern their movement into the vial.

However, the reliability of this sampling method was tested because of the possibility that differences in activity or in response to light either between the sexes or between mutant types might result in biased samples. In order to make this test, eight populations of glass versus wild type were sampled in the usual manner and then the rest of the flies were removed from the bottles and also counted. The results in table 1 show that there was no significant difference between the sampled flies and the remaining flies with respect to either sex ratio or frequency of the mutant types. These results do not completely eliminate the possibility that bias existed when other alleles were involved. However, they do show that when a highly disadvantageous mutant (see below) affecting the eyes was sampled, the samples obtained were not grossly biased, either with respect to sex ratio or frequency of the mutant type.

One set of experiments involved a group of genes at or near the white locus on the X chromosome. The mutants used were white, apricot, coral, eosin, and satsuma.

TABLE 1

Comparison of samples with remaining adult populations in 8 populations of glass vs. wild type

Population	Sample			Remainder		
	Males	Females	*gl/gl*	Males	Females	*gl/gl*
1	18	23	0	63	67	3
2	38	24	0	85	87	0
3	11	11	1	23	17	0
4	25	37	0	99	94	0
5	55	58	0	96	115	0
7	57	72	0	62	49	2
9	35	40	0	77	80	0
10	12	24	0	33	65	1
Totals.....	251	289	1	538	574	6
	% males = 46.5% $P = .3$			% males = 48.4% $P > .3$		
	% *gl/gl* = 0.19% $P = .16$			% *gl/gl* = 0.54% $P > .3$		

LEWIS (1952) has shown white and apricot to be pseudoalleles, and MACKENDRICK and PONTECORVO (1952) have shown white and coral to be pseudoalleles, but did not find crossovers between white and satsuma, eosin and apricot, or eosin and satsuma. Both coral and apricot belong to the so-called "apricot" group in which the eye color is darker in the male than in the female and the Pale modifier darkens the eye color. (However, it should be noted that MULLER (1950) states that no certain difference in eye color can be distinguished between the apricot males and females.) White and eosin belong to the "eosin" group, which shows a lighter eye color in males than in females, and in which the Pale modifier lightens the eye color. Satsuma shows no sexual dimorphism and is not modified by Pale.

The white stock was made relatively isogenic by single pair, brother-sister matings for 12 generations. During this period of inbreeding, the other four mutant stocks were repeatedly backcrossed to white so that by the end of this time all five stocks should have been rather similar genetically except at the mutant locus and probably closely adjacent regions. The backcrossing was carried out by first single pair mating a male sib from the white line to an eosin female, for example, and then backcrossing a heterozygous w^e/w female to a white male sib from the next inbred generation of white.

After the inbreeding, 5 populations of each mutant in competition with white were started, with 10 mutant males, 10 white males, and 10 heterozygous females as the initial population, the genes thus being present with equal frequency at the outset. The populations were sampled at intervals roughly of 30 days. Since the heterozygous females can be distinguished from the homozygotes, except for heterozygous white-satsuma females, it was possible to determine the exact gene frequencies in the samples at all times.

Another set of experiments involved competition between sex-linked and autosomal recessive genes and their wild type alleles. The mutants used were the sex-linked

recessives, yellow (*y*) and raspberry (*ras*^{2}), and the autosomal recessives, vestigial (*vg*) and glass (*gl*). Ten populations of each recessive mutant were started from laboratory stocks. After these populations were well established and were estimated to consist of over 200 adult flies, a single freshly emerged wild type (Lausanne Special) male was introduced into each population on the 20th day after the mutant population had been started. The male was placed in the slant of fresh food and allowed to recover completely from the ether before this bottle was connected to the other by means of the rubber hose. The initial frequency of the wild type allele at the time of introduction of the male was then, at a maximum, about 0.005, and was probably less in most cases.

A comparison was made of the viability of the mutant types and their heterozygous wild type sibs by counting the progeny from a backcross of heterozygous females to mutant males reared in half-pint culture bottles under the conditions given above. Mating tests of the "female choice" type (MERRELL 1949, 1950) were conducted by placing 10 females in a creamer with 10 mutant and 10 wild type males, all of these flies being the progeny of a backcross of heterozygous females to mutant males. After 2 days, each of the females was isolated in a creamer, and her progeny were examined to determine the genotype of the father.

RESULTS

The white series

The changes in gene frequency in the populations can be followed in figures 1, 2, 3, and 4 (the complete data on which these figures and figures 5 through 8 are based are on file in the GENETICS editorial office). With the sex-linked genes only the changes in the males are shown because they adequately reflect what happened in the populations. Figures 1 through 4 show that rapid, consistent shifts in gene

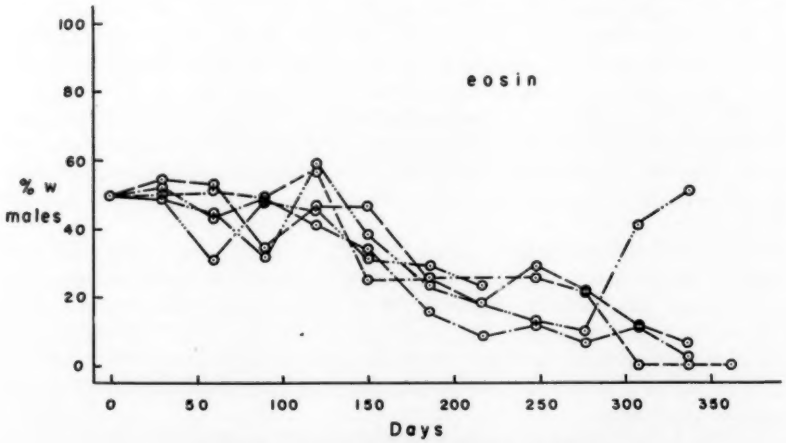

FIGURE 1.—Competition between eosin and white.

frequency, attributable to strong selection pressure against one of the genes involved, do not occur. However, trends do appear in most cases, suggesting that the genes are not completely neutral in effect.

The frequency of the genes tends in most cases to differ between males and females (tables 2 and 3). Only in the satsuma group is there no evidence of differences. In the apricot group, when individual samples were compared, no significant difference emerged, but when the totals for each population were examined, the frequency of *w* was higher in the males than in the females in all five populations.

The expected numbers of heterozygous and homozygous females were calculated for each sample from the Hardy-Weinberg formula and from the observed gene fre-

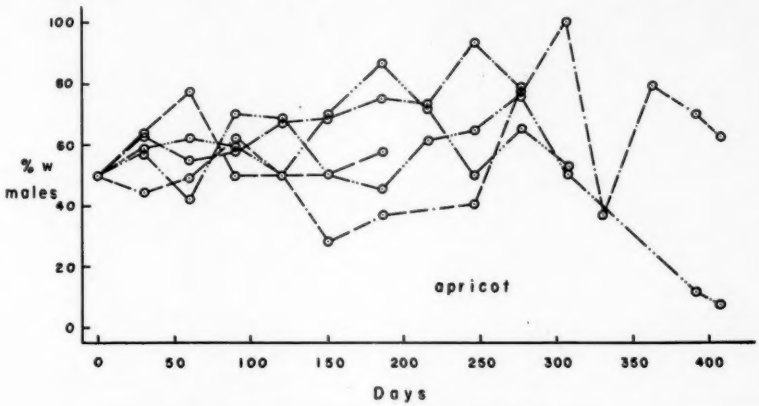

FIGURE 2.—Competition between apricot and white.

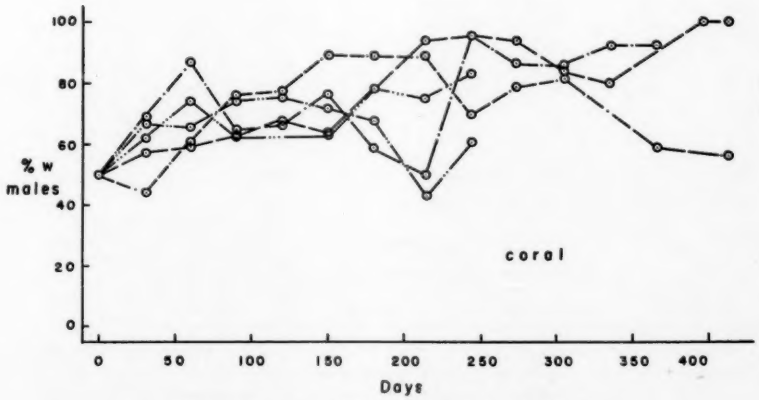

FIGURE 3.—Competition between coral and white.

quencies in the females, and compared with the observed numbers. No comparison could be made involving satsuma because w^{sat}/w females were indistinguishable from w^{sat}/w^{sat} females. In the populations of eosin and white, the individual samples did not differ significantly from the expected numbers, but observed numbers were

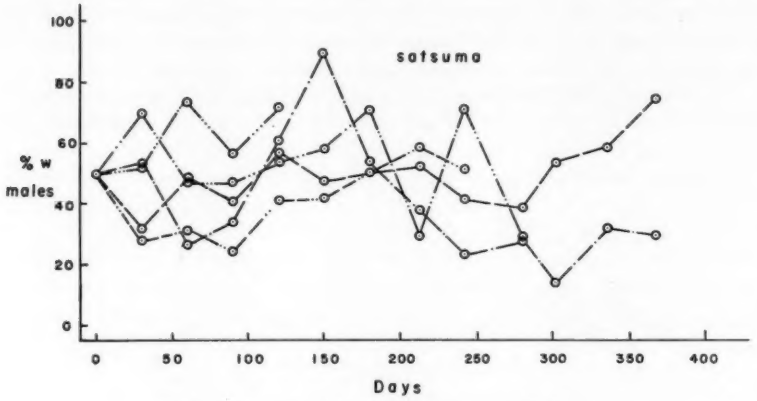

FIGURE 4.—Competition between satsuma and white.

TABLE 2

Comparison of the frequency of w *in males and females*

Population	w^e-w %w		apr-w %w		w^{co}-w %w		w^{sat}-w %w	
	Males	Females	Males	Females	Males	Females	Males	Females†
1	36.6	42.0	62.6	47.8	73.8*	65.6*	48.0	49.2
2	28.2	30.7	67.8	66.7	78.6	66.5	38.9	36.6
3	39.8	46.3	47.4	41.8	68.0	49.1	53.5	35.2
4	31.3	39.9	57.9	52.4	68.1	56.9	38.7	31.6
5	38.0	39.2	66.0	63.2	68.3	56.8	65.4	64.5
	34.8	39.7	58.7	52.6	71.9*	59.6*	47.1	42.3

* Omitting last two samples in population 1 when $w = 100.0\%$.

† Estimated from frequency of w/w females.

TABLE 3

Comparison of the frequency of w *in males and females*

Populations	Samples compared	$q_f > q_m$	$q_f < q_m$	x^2
w^e-w	46	38	8	19.56†
apr-w	47	22	25	0.19
w^{co}-w	49	13	36	10.80†
w^{sat}-w	35	17	18	0.03

† Highly significant.

greater than expected somewhat more often than they were less (table 4). However, highly significant excesses of heterozygous females were observed in the apricot-white and coral-white populations.

The sex ratios deviated from equality in these populations (table 5). In all cases

TABLE 4

Comparison of observed and expected frequency of heterozygous females

Populations	Samples compared	$O > E$	$O = E$	$O < E$	χ^2
w^a-w	46	30	0	16	4.26*
apr-w	48	44	0	4	33.34†
w^{co}-w	48	33	3	12	9.80†

* Significant.

† Highly significant.

TABLE 5

Sex ratios in populations containing members of the white series

Population	w^a-w		*apr*-w		w^{co}-w		w^{sat}-w	
	Total of samples	% males	Total of samples	% males	Total of samples	% males	Total of samples	% males
1	1326	61.4	370	51.4	1127	51.4	752	66.0
2	811	61.3	653	55.6	861	60.8	684	67.2
3	670	61.8	1177	48.3	571	59.2	711	64.7
4	896	55.7	831	53.2	314	63.0	682	67.7
5	717	63.9	698	54.0	1176	56.4	342	68.4
	4420	60.7	3729	52.0	4049	56.8	3171	66.6

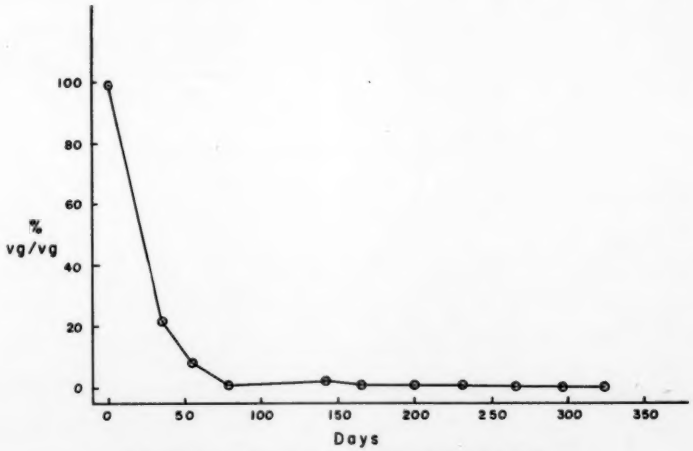

FIGURE 5.—Competition between vestigial and wild type.

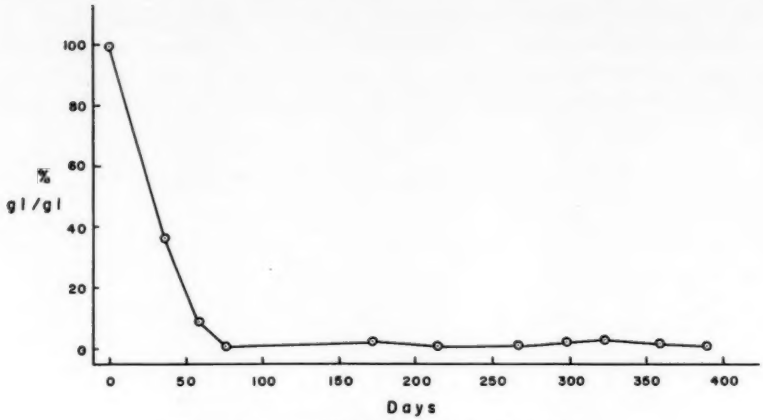

FIGURE 6.—Competition between glass and wild type.

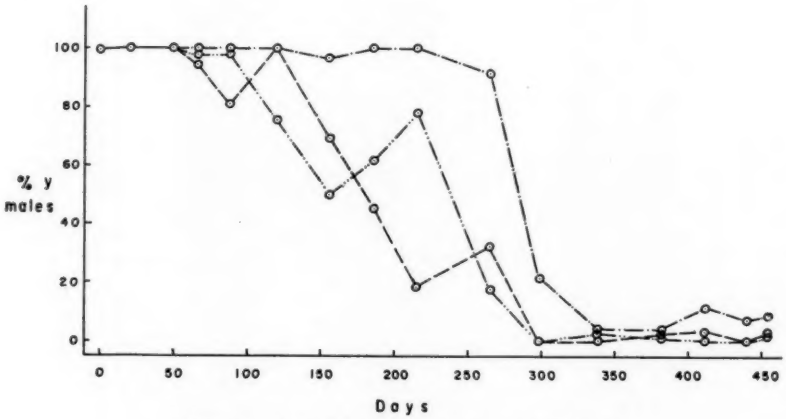

FIGURE 7.—Competition between yellow and wild type.

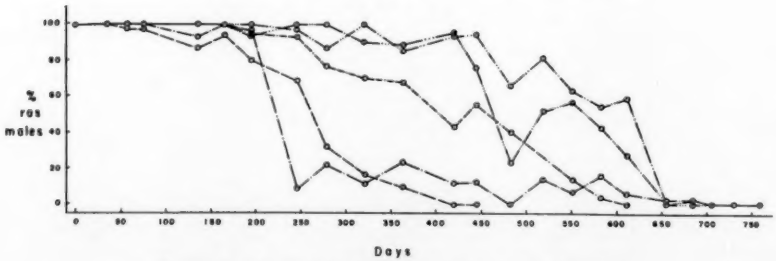

FIGURE 8.—Competition between raspberry and wild type.

TABLE 6

Comparison of viability of mutant and wild type sibs

Genotype	+/*gl*	*gl*/*gl*	+/*vg*	*vg*/*vg*
Males	795	723	863	802
Females	822	745	962	851
Total	1617	1468	1825	1653
	1.000	.908	1.000	.906
Genotype	**+ or +/*ras***	***ras* or *ras*/*ras***	**+ or +/*y***	***y* or *y*/*y***
Males	1476	1420	444	477
Females	1399	1418	475	440
Total	2875	2838	919	917
	1.000	.987	1.000	.998

TABLE 7

Female "choice" mating tests

Female Genotype	Total females	Successful males		Double matings
		Wild type	Mutant	
A. Equal numbers of mutant and wild type males				
+/*y**	73	62	3	1
y/*y**	97	68	8	1
Total	170	130	11	2
+/*ras**	68	40	21	0
ras/*ras**	76	39	18	1
Total	144	79	39	1
+/*gl*	49	26	1	0
gl/*gl*	48	30	0	1
Total	97	56	1	1
+/*vg*	48	42	4	1
vg/*vg*	48	41	4	0
Total	96	83	8	1
B. 18 mutant to 2 wild type males				
gl/*gl*	43	22	12	0
vg/*vg*	41	15	22	0

* MERRELL 1949.

TABLE 8

Sex ratios in populations with vg, gl, y *or* ras

Type of population	Total of samples	% males
A. Mutant and wild type		
vg—10 populations	7281	50.5
gl—9 populations	6537	45.9
y—3 populations	3381	46.3
ras—5 populations	7726	48.9
B. Mutant only		
gl—1 population	328	36.0†
y—6 populations	2533	41.8†
ras—5 populations	3125	47.4†

† Highly significant difference from sex ratio in comparable populations under A.

there was a significant excess of males, the satsuma-white populations showing the greatest deviation, 2 males to 1 female. Although there were fluctuations in the ratio from sample to sample, each group of populations appears to have a more or less characteristic sex ratio.

Wild type vs. mutants

The changes in frequency of the mutant types, *vg*, *gl*, *y*, and *ras*, in competition with the wild type are shown in figures 5, 6, 7, and 8. The changes in frequency were so uniform in the ten populations of *vg* and nine of *gl* in which the wild type became established that the data have been combined to give a single curve in each case. In each of these 19 populations the frequency of the mutant phenotype had dropped from more than 99% at the outset to less than 10% by the 55th day. The course of events in the populations with *y* and *ras* can be followed from the changes in gene frequency observed in the males. However, each population is shown separately because the course of events in each was unique. The wild type became established in all ten *vg* populations, and nine of ten *gl* populations, but only in three out of nine *y* populations, and five out of ten *ras* populations.

The outcome of the tests for viability and mating success is shown in tables 6 and 7. The mutants appear to have a more drastic effect on mating success than on viability under the conditions of the experiments.

The sex ratios in the various populations are shown in table 8. A comparison has been made between the sex ratios in the populations in which the wild type became established and those populations remaining homozygous for the mutant.

DISCUSSION

The w series

There was no great difference in selective value among the alleles and pseudoalleles of white. Rapid changes in frequency, usually observed in competitions between wild type and mutant alleles, did not occur. These *w* genes presumably have never been in competition before. Nevertheless, the indications are that they can not be considered to be altogether adaptively neutral. Trends were observed in three of the

four sets of experiments. Only w^{sat} and w appeared to be comparable to each other and under different conditions they might also show selective differences. It would be interesting to know whether, in a more heterozygous background, modifiers would accumulate due to selection which would cause one gene to become decidedly superior to the other.

However, although the genes did not change frequency rapidly, they apparently differed in their effects in the males and females. A sex ratio of 1:1 was approached most closely in the w vs. apr populations, apparently due to heterosis in the $apr +/+ w$ females. A general statement could be made that the females, with two doses of any of these genes, were less viable than the males, with only a single dose.

This finding raises certain questions about the theory of dosage compensation advanced by MULLER (1932, 1950). Since males and females usually show rather similar phenotypes for traits controlled by sex-linked genes despite two doses of these genes in females and only one in males, MULLER has suggested that the differences in gene dosage have in some way been compensated. This dosage compensation is presumed to be due to the action of modifiers in the X chromosome, an intrachromosomal balance being achieved between the mutant and the modifiers. He further suggests that the dosage compensation system functions primarily to make the effects of the wild type genes the same in both sexes. The system of modifiers is thought to have evolved in relation to the wild type, but shows up best in the presence of hypomorphic mutants.

Since the expression of the eye mutants in these populations showed no apparent differences from the usual descriptions (BRIDGES and BREHME 1944), with slight differences or none between hemizygous males and homozygous females, presumably a normal system for dosage compensation was present. However, if such modifiers adjust a trait like eye color to more or less comparable values in males and females, then they would also be expected to adjust the viability of the males and females to comparable levels (see discussion below on the sex ratio). Furthermore, these modifiers might be expected to produce comparable viabilities as well as eye colors even if the differences in viability were due to sex-linked genes other than the w series itself. However, the most extreme sex ratio, 2 males:1 female, occurred in the satsuma-white populations where there was no sexual dimorphism for either satsuma or white. Hence, it seems clear that the expression of each trait, eye color and viability, was independent of the other, whether due to pleiotropic effects of the w genes or to the separate action of the w series on eye color and of other sex-linked genes on viability. In either case, if dosage modifiers were present, they would be expected to have modified both eye color and viability under MULLER's hypothesis. Hence, the suggestion (GOLDSCHMIDT 1938, 1954) that similarities in eye color are due to a threshold effect during development may be more useful in this instance since under this hypothesis, it is possible for eye colors to be similar but viabilities different when there are differences in gene dosage.

It might be expected that the most pigmented eye would be the best adapted, but this is not so. The sequence of genes from best adapted to least adapted is not the same as the sequence from most pigmented to least pigmented. The adaptive relations are roughly as follows: $w^e > w \cong w^{sat} > apr > w^{co}$, while the sequence

with regard to amount of pigment is $w^{sat} > w^{co} > w^{e} > apr > w$. Similar results have been reported by TIMOFÉEFF-RESSOVSKY (1933). These differences may be due to other viability genes closely linked to the *w* locus, but they may also be due to the effects of the mutants themselves. If so, the mutants' effects on eye color are only one aspect of their overall selective value.

The frequency of the less favored allele was generally lower in the males than in the females. Thus in w^{e} vs. *w* populations, the less favored *w* was less frequent in males than in females, whereas in w^{co} vs. *w* populations, the less favored w^{co} was less frequent in males than females. No difference was found in the w^{sat} vs. *w* or the *apr* vs. *w* populations between frequencies in males and females; in w^{sat} vs. *w* apparently because of the equivalence in adaptive value, in *apr* vs. *w* probably because of the heterosis involved in the females. This result is in accord with expectation since there are only two types of males, but three types of females. Selection can thus be expected to be more effective in the males because they do not carry the less favored gene in the heterozygous condition.

This result presents an apparent paradox since selection seems to be more effective in the males, but the total numbers of males exceed the totals for the females. Actually selection can be thought of as being more discriminating in the males than in the females, gametic selection being more effective than zygotic (which is essentially the difference here). This effect, then, is in addition to the effects of dosage discussed above.

The heterosis observed in females heterozygous for white and either of its pseudoalleles, apricot or coral, and possibly for white and eosin, requires comment. Heterosis has also been reported for the pseudoalleles in the lozenge region in *Drosophila melanogaster* by OLIVER and GREEN (1944). These authors ascribed the heterosis to interaction between the lozenge genes. Another possibility is that the heterosis is due to the presence of a wild type allele at each locus. Still another explanation may be that the heterosis is due to residual differences at loci closely linked to the mutants which did not become uniform during the backcrossing to white. If the latter is the case, it is a rather interesting coincidence that the known pseudoalleles should produce the maximum heterosis while eosin showed little. If the heterosis is due to the genes within the white region, then the second explanation, that heterosis is due to the presence of a wild type allele at each locus, seems more probable than the first. If heterosis is, indeed, characteristic of pseudoalleles, and if pseudoallelism is as common as recent studies (in Drosophila, LEWIS 1952; GREEN and GREEN 1949; IVES AND NOYES 1951; MACKENDRICK and PONTECORVO 1952; in mice, DUNN and CASPARI 1943; in Neurospora, GILES 1951; BONNER 1951; and in Aspergillus, PONTECORVO 1953) seem to indicate, this relationship may have considerable importance. It lies in the fact that new phenotypes may be exposed to selection without being completely divorced from the adaptive value of the wild type.

Furthermore, those cases of heterosis which have been reported as involving alleles at a single locus may be indicative of pseudoallelism. For instance, the marked heterosis noted by KERR and WRIGHT (1954) in heterozygotes of spine-

less (*ss*) and aristapedia (ss^a) compared to both homozygotes may indicate that they are pseudoalleles, a possibility made even more likely by the rather different nature of their morphological effects. Also, when interallelic interaction has been reported as supporting evidence for the overdominance theory of heterosis (SINGLETON 1943; GUSTAFSSON 1952; and others), the heterosis may actually have been due to pseudoallelic interaction interpretable in terms of the dominance hypothesis.

Wild type versus mutants

In contrast to the results with the *w* series, the wild type genes showed a decided superiority over their mutant alleles. The changes in frequency of the *vg* vs. + and *gl* vs. + populations were characterized by an initial precipitous drop in the frequency of the mutant type followed by a more gradual decline. Although a similar leveling off has been interpreted to indicate that an equilibrium has been attained when the mutant reached a low frequency (L'HERITIER and TEISSIER 1937), the rate of elimination is so slow at low frequencies that the decision as to whether or not equilibrium has been reached is very difficult unless the experiment is continued for a long period. Although the viability of the mutants is fairly high, about 90% that of the wild type under favorable conditions, the clue to their low adaptive value apparently lies primarily in their effect on mating behavior. It should be noted that the mode of decline in frequency of the mutants in these populations fits the type of change theoretically expected when there is complete selection against an autosomal recessive gene.

In those *y* vs. + and *ras* vs. + populations in which the wild type gene became established, the decline in frequency of the mutant type was more gradual than in the populations carrying the autosomal recessives. The most rapid shifts in frequency of the mutant type came at intermediate frequencies, with the changes being more gradual at both high and low frequencies of the mutant type. This finding is in accord with the predictions of population genetics, which have indicated that selection is most effective at intermediate gene frequencies. Again the mating behavior seems very closely tied to the results in the populations. When tested, neither *y* nor *ras* showed significant differences from the wild type as measured by the number of adults produced, but both reduced the mating efficiency of the males, *y* considerably more than *ras*. The more rapid decline of *y* in the populations in which the wild type became established as compared to *ras* indicates agreement between the mating tests and the changes in the populations.

On the basis of the tests made of viability and mating success, the mutants compared to each other could be rated as follows: all + alleles > *ras* > *y* > *vg* ≅ *gl*. These ratings are borne out by the relative success of the mutants in the populations, for the rate of change in frequency in the populations is much slower for *ras* than it is for *vg* or *gl*, where the selection coefficient is so much greater. A further prediction of population genetics especially emphasized by WRIGHT is that when selection is weaker, chance events play a greater role in the course of events in the populations. This prediction is borne out in a crude way by the fact that in the *y* vs. + and *ras* vs. + populations, the wild type became established in only 8 of 19

populations while it became established in 19 of 20 *vg* vs. + and *gl* vs. + populations. Hence, where the selective advantage of the wild type gene was less, it had a lower probability of becoming established in the population.

Sex ratios

The sex ratios involved in the wild type-mutant competitions showed an excess of females over males or else a 1:1 sex ratio in contrast to the results with the *w* series where males outnumbered females. Since all of the populations were sampled in the same fashion, it becomes unlikely in view of these results that sex was responsible for sampling bias. Other authors have reported that in *Drosophila melanogaster* populations they have usually obtained an excess of females over males (MOENKHAUS 1911; REED and REED 1950; LUDWIN 1951). A further observation from the above data is that the sex ratio in populations containing both the wild type and the mutant type is generally closer to 1:1 than is the ratio in homozygous mutant populations (table 8). These results can be interpreted to indicate that the various mutant genes were more deleterious in one sex than the other. The wild type genes, on the other hand, came closer to producing equal viability in both sexes. If such is generally the case, then presumably the secondary sex ratio has been adjusted by natural selection toward unity in the adults. FISHER (1930a) and SHAW and MOHLER (1953) have reasoned that natural selection should always act toward producing equality of the sexes at maturity. These results would appear to bear out their conclusion. However, no answer can be suggested as to whether the equality produced is due to the selection of modifying factors or to the selection of those among the wild type isoalleles which have equivalent effects in males and females.

Since these genes do affect the sex ratio so markedly, their adaptive values must be different in males and females. This conclusion indicates the possibility, as FISHER (1930b, 1932) has suggested, that balanced polymorphism could arise because a mutant which is advantageous in one sex may be disadvantageous in the other. This suggestion has been more or less neglected in the more recent discussions of the mechanism of balanced polymorphism (DOBZHANSKY 1951; FORD 1953), which have emphasized the superiority of the heterozygote over both homozygotes, fluctuating selection pressures, or ecological differences as instrumental in producing balanced polymorphism. The above data do not demonstrate this type of balanced polymorphism, but they do demonstrate the validity of the basic assumption, that the same gene may have different adaptive values in males and females. LEVITAN (1951) has presented evidence which indicates that there are differences in the adaptive value of different gene arrangements in the males and females of *Drosophila robusta*. Therefore, the polymorphism in gene arrangements, common to many species of Drosophila, could also be due to this cause as well as to adaptively superior heterozygotes.

SUMMARY AND CONCLUSIONS

1. Competition between alternative genes was followed in experimental populations of *Drosophila melanogaster*. In one set of experiments, white was placed in

competition with coral, eosin, satsuma, or apricot. Another set involved the sex-linked recessives yellow (*y*) and raspberry (*ras*), and the autosomal recessives vestigial (*vg*) and glass (*gl*), each in competition with its respective wild type allele.

2. In the experiments with white, the genes used came closer to being adaptively neutral than any tested thus far, but even so, trends were noted in favor of one or the other gene in all of the competitions except white vs. satsuma. Hence, the concept of the completely neutral gene pair is not generally supported by these results.

3. In the white vs. apricot and white vs. coral experiments there was a decided excess of heterozygous over homozygous females. In the white vs. eosin experiments there was possibly a slight excess of heterozygous females, but not to the degree seen with apricot and coral. Since apricot and coral have been shown to be pseudo-alleles of white, this heterosis may be due to the respective wild type alleles of these genes. These results further suggest the possibility that instances of heterosis reported as due to the interaction of alleles to produce overdominance may be of this kind.

4. The overall sex ratio in the white populations showed an excess of males over females, the highest being 2 males: 1 female in white-satsuma. This result is interpreted as being due to two doses of these deleterious genes in the females as compared to only one in the males. Since the effect of these genes on viability appears to be unrelated to their effect on eye color, the theory of dosage compensation for sex-linked genes does not seem to apply in this case.

5. The decline in frequency of the autosomal recessives, *vg* and *gl*, approximates the theoretical curve expected for complete selection against an autosomal recessive. The primary selection pressure was apparently not due to loss of viability, which was approximately 90% of the wild type on test in both cases, but rather to the reduction of mating success by the mutant males.

6. The decline in frequency of the sex-linked recessives, *y* and *ras*, was more gradual. The prediction from theory that the most rapid decrease in frequency of the less favored allele should occur at intermediate gene frequencies, with the decline more gradual at both high and low frequencies of the mutant, was borne out experimentally. Again the selection pressure was apparently due to reduced mating success rather than loss of viability.

7. The overall sex ratio in populations in which the wild type became established came closer to equality than the ratio in those populations which remained homozygous for the mutants. This result supports the theoretical conclusion that natural selection always tends to shift the secondary sex ratio in the direction of equal numbers of both sexes.

8. The variety of sex ratios observed indicates that genes may have different selective values in males and females. This observation suggests a further mechanism for balanced polymorphism.

9. The relationship between strength of selection pressure and chance events was demonstrated by the fact that when selection against the mutants was strong, as in *vg* and *gl*, the favored wild type became established in 19 of 20 populations. On the other hand when the selective advantage of the wild type was not so great, it was lost from 11 out of 19 *y* and *ras* populations.

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NEW ASPECTS OF THE BALANCED LETHAL MECHANISM IN OENOTHERA[1]

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Received January 10, 1956

CERTAIN kinds of Oenothera are well known as naturally occurring translocation heterozygotes whose heterozygosis is maintained through successive generations by self-pollination and a balanced lethal mechanism. In recent years, especially through the work of CLELAND and his associates, natural populations of Oenothera have been analyzed cytogenetically for chromosomal end arrangement to obtain evidence of their phylogenetic relationships (see CLELAND, *et al.* 1950). In connection with such studies, an investigation has been undertaken by the author to determine whether members of a particular phylogenetic grouping carry the same lethal factors, and whether, therefore, lethals can be used as an additional index of phylogenetic relationship. The results of this investigation, which has been of an exploratory nature, reveal some interesting and new aspects of the balanced lethal mechanism in Oenothera which seem of sufficient interest to warrant a preliminary report; complete analysis will require several additional generations

The phylogenetic grouping selected for this study is one which has been tentatively designated by CLELAND as the "biennis group 1". In some respects at least, its members show a much closer relationship to one another than is true in most of the other groups which have been studied; thus it did not seem unlikely that some of the forms might possess identical lethal factors. The geographic range of the biennis group 1 extends from Missouri and Arkansas throughout the central Midwest to the East Coast in the region of Virginia and southward into a large part, if not most of the Southeast. Plants belonging to this category are typically structural or "complex"-heterozygotes showing a circle of fourteen chromosomes at meiosis. Adjacent chromosomes of the circle regularly go to opposite poles so that only two kinds of genomes or "complexes", each with a different chromosomal end arrangement, occur among the spores. Although these forms are normally self-pollinating, homozygous combinations of the complexes do not occur among their progenies. When there is evidence from outcrossing that both complexes occur among the viable pollen as well as the eggs, the failure of the homozygotes to appear has been attributed to zygotic lethals. RENNER (1917, 1925, 1933) has shown this to be the case in *Oenothera lamarckiana* as well as many other forms. There are other races which, as far as one can determine from outcrosses, transmit only one of the two complexes present in the heterozygote through the pollen, and the other only through the egg. This has been interpreted as evidence of gametophytic lethals.

Situations intermediate between the two outlined above are also found; in fact,

[1] Work supported by the Faculty Research Fund of the Horace H. Rackham School of Graduate Studies, University of Michigan. A portion of this project was carried out during tenure of a Summer Faculty Fellowship.

the majority of the races of the biennis group 1 behave in this way. Some races in outcrosses transmit both complexes through the egg and only one through the pollen. In others, both complexes may come through the pollen and only one through the egg. Furthermore, in different outcrosses the percentage of progeny which receives a particular complex may vary widely. In this paper the convention will be followed in which the complex which is transmitted entirely or predominantly through the egg is called the alpha complex, and the beta complex designates the genome which comes mainly through the pollen.

The races of the biennis group 1 are characterized by possessing alpha complexes having for the most part identical arrangements of chromosome ends. Of the forty-two alpha biennis group 1 complexes so far analyzed, thirty-two possess the chromosome end arrangement, 1·2 3·4 5·14 7·10 9·8 11·12 13·6.[2] Moreover, the alpha complexes of this group all show basic phenotypic similarities such as broad, thin, dark green, crinkly leaves and thin-sepaled, glabrous flower buds with clasping bracts. The beta complexes of the biennis group 1 races display, in contrast, the strigosa phenotype, characterized by thick, narrow, grayish-green leaves and buds with a heavy appressed pubescence. Their segmental arrangements are much more variable than those of the alpha complexes. The alpha complexes are unusual in their segmental and phenotypic uniformity.

Evidence regarding the identity of zygotic lethals among the alpha biennis 1 complexes can be obtained from the results of crosses between different biennis group 1 races. If among the progeny of such crosses viable hybrids combining the alpha complex of each parent occur, the conclusion follows that the two alpha complexes do not possess identical zygotic lethal factors. This is pointed out by RENNER (1925): "Durch Kreuzung zweier komplexheterozygotischer Arten ist eine homozygote Sippe natürlich nur dann zu gewinnen wenn ihre Letalfaktoren nicht homolog sind." Alpha·alpha combinations can be expected, of course, only in crosses in which the pollen parent is known to be capable of producing functional pollen carrying the alpha complex; races of this type cannot possess a male gametophytic lethal. Thus, the method outlined here can be expected to give only information about zygotic lethals. Nevertheless, evidence regarding the identity of zygotic lethals among the alpha biennis 1 complexes, which on the basis of identical segmental arrangements and similar phenotypic effects are closely related, might give insight into the phylogeny of the biennis group 1.

Alpha·alpha combinations in which each complex possesses the same segmental arrangement show at meiosis seven pairs of chromosomes. It was hoped that through inbreeding the structural homozygotes obtained from these crosses, it would be possible to select among succeeding generations segregates in which lethals were no longer present. Thus we would have obtained from a complex-heterozygote a derivative with a return to the original type of chromosome behavior. This would be of interest in the light of STINSON's (1953) conclusion that such derivatives are not

[2] Several hundred collections of North American Oenotheras have been analyzed and the end arrangements of their complexes determined in terms of a standard arrangement. Many of these determinations, carried out by CLELAND and his associates, have not been published. For further details regarding the methods of analysis, see CLELAND *et al.* 1950.

likely to occur in nature. It would also indicate that the highly specialized complex-heterozygote still retains some evolutionary plasticity.

MATERIALS AND METHODS

The races of the biennis group 1 used in this study are the following:

Race	Geographic origin
Baltimore	Maryland
Birch Tree 1	Missouri
Birch Tree 2	Missouri
Camp Peary E	Virginia
Camp Peary L	Virginia
Friendship	Indiana
Hopkinsville	Kentucky
Hot Springs	Arkansas
Lake	Virginia
Magnolia	Kentucky
Paducah	Kentucky
Princeton	Indiana
Rich Mountain	Arkansas
Warwick A	Virginia
Warrenton	Virginia
Williamsburg	Kentucky
Winslow	Arkansas

The alpha complexes of these races all possess the typical alpha biennis 1 chromosome end arrangement. Thus hybrids between the races which carry the alpha complex of each parent must show seven pairs of chromosomes at meiosis. In order to obtain an alpha·alpha hybrid, the race used as the male parent must, of course, transmit the alpha complex (normally the egg complex) through the pollen. Where earlier data indicated conclusively that a race did not transmit the alpha through the pollen, the race was not used as male parent in any of the crosses. Where previous data were limited, crosses were made to determine whether a particular race transmitted the alpha complex only through the egg.

A preliminary series of biennis group 1 hybrids was studied during the summer of 1952. A much more extensive set of crosses was grown in 1955. The size of the hybrid progenies ranged from five to sixty-nine plants, although most cultures in the first series consisted of fifty or more plants, while in the second series the majority of cultures contained from thirty-five to forty individuals. All cultures were observed for the appearance of distinct classes at the seedling stage, at the rosette stage, and periodically after being placed in the field. Identifications of the different hybrid combinations among the progeny were made on the basis of phenotype and confirmed by cytological observations.

Controlled self-pollinations were carried out on the majority of the cytologically confirmed alpha·alpha combinations of both series. The flowers of the alpha·alphas

proved to be naturally self-pollinated; therefore, an entire inflorescence was simply bagged and the bag allowed to remain long enough for ten to twenty flowers to undergo pollination. As an added safeguard, a few flowers of each alpha·alpha were also pollinated by hand.

EXPERIMENTS AND RESULTS

The results of all crosses between biennis 1 races are shown in table 1. Part A includes the first series studied in 1952, while Part B consists of those analyzed during the summer of 1955. The races Baltimore, Camp Peary L, Friendship, Hopkinsville, Magnolia, Princeton, Rich Mountain, Warrenton, Williamsburg and Winslow do not transmit the alpha complex through the pollen. Crosses were made with some of these races as pollen parents, but since they have no pertinence to the problem other than to establish that the alpha complex does not come through the pollen, they are not listed in table 1. The races Lake and Warwick A transmit their alpha complexes through the pollen only rarely. Of a total progeny of 459 obtained in crosses with Lake as the male parent, only two alpha·alphas occurred, while Warwick A used as a pollen parent gave only one alpha·alpha among 502 offspring. Crosses with these races as pollen parents have not been included in table 1.

Structural homozygotes of alpha·alpha constitution appeared among most of the crosses listed. In the 1955 series, for example, Hot Springs transmitted the alpha complex through the pollen to some of the progeny in every cross, Camp Peary E in all except one cross, Birch Tree 2 in every cross except two, and Birch Tree 1 in all but three crosses. In crosses with Paducah as pollen parent, however, seven out of fifteen cultures failed to show alpha·alpha combinations. Table 2 summarizes the data for each of these races with regard to the frequency of alpha·alphas among their total progenies.

Identifications of the alpha·alpha combinations could be made with certainty in most cases from the phenotype of the mature plants. The glabrous buds and clasping bracts serve as the most definitive characters; in addition, the structural homozygotes tend to possess somewhat thinner, broader leaves than the heterozygotes. Although recognition of the alpha·alpha combinations in general presents no difficulties, the differences between a plant with two biennis-type complexes (alpha·alpha) as contrasted with one carrying a biennis and a strigosa-type complex (alpha·beta) are not always as striking as might be expected. In many cultures the classes could be distinguished shortly after the rosettes were growing in the field. The distinctive characteristics of the alpha·alpha combinations at this stage in contrast to the alpha·betas are the darker green, broader, more strongly crinkled, but less wavy leaves often showing bronze margins and tips. These rosettes were usually slower to develop than the alpha·betas. In some cases an approximately correct classification was possible while the plants were still in the greenhouse. Many of the alpha·alphas were recognizable at an early stage because they were chlorotic. This characteristic was not reliable, however, since usually it was not found in all of the alpha·alphas of any one culture; alpha·betas of some cultures showed it as well.

Cytological confirmation was made in all except nine of the seventy-four different alpha·alpha combinations obtained. In all cases they showed regular pairing at

TABLE 1

Part A.—Occurrence of alpha·alpha complex combinations among crosses between different races of biennis group 1

Culture no.	Cross	α·α	α·β	β·β	Total progeny	% α·α	Cytologically confirmed
87	Birch Tree 1 × Birch Tree 2	—	43	—	43	0	
88	Birch Tree 1 × Hot Springs	1	34	—	35	2.8	+
90	Warrenton × Birch Tree 1	12	28	—	40	30	+
91	Warrenton × Birch Tree 2	15	35	—	50	30	+
94	Warrenton × Hot Springs	31	19	—	50	62	+
97	Friendship × Birch Tree 1	36	30	1	67	54	+
98	Friendship × Birch Tree 2	13	54	—	67	19	+
100	Friendship × Hot Springs	40	28	—	68	59	+
104	Birch Tree 2 × Birch Tree 1	—	36	—	36	0	
107	Birch Tree 2 × Hot Springs	—	57	—	57	0	
110	Hopkinsville × Birch Tree 1	39	21	—	60	65	+
111	Hopkinsville × Birch Tree 2	2	63	—	65	3	+
113	Hopkinsville × Hot Springs	38	28	—	66	57	+
115	Hot Springs × Birch Tree 1	38	19	—	57	67	+
116	Hot Springs × Birch Tree 2	23	33	—	56	41	+
118	Paducah × Birch Tree 1	4	1	—	5	80	+
119	Paducah × Birch Tree 2	1	62	—	63	1.5	+
122	Paducah × Hot Springs	42	27	—	69	61	+
124	Winslow × Birch Tree 1	55	12	2	69	80	+

Part B.—Occurrence of alpha·alpha complex combinations among crosses between different races of biennis group 1

100	Baltimore × Birch Tree 1	27	13	2	42	64.2	+
102	Baltimore × Birch Tree 2	4	31	3	38	10.5	+
103	Baltimore × Camp Peary E	18	15*	5	38	47.5	+
105	Baltimore × Hot Springs	20	16	0	36	55.5	+
107	Baltimore × Paducah	9	29	0	38	23.6	+
111	Birch Tree 1 × Birch Tree 2	0	21	0	21	0	
112	Birch Tree 1 × Camp Peary E	1	19	0	20	5.0	+
114	Birch Tree 1 × Hot Springs	7	24	0	31	22.6	+
116	Birch Tree 1 × Paducah	2	37	0	39	5.1	+
120	Birch Tree 2 × Camp Peary E	0	14	1	15	0	
121	Birch Tree 2 × Hot Springs	6	16*	0	22	27.2	+
122	Birch Tree 2 × Paducah	2	34	1	37	5.4	+
123	Camp Peary E × Birch Tree 1	14	21	0	35	40.0	+
124	Camp Peary E × Birch Tree 2	25	12	1	38	66.0	+
127	Camp Peary E × Hot Springs	30	7	0	37	81.1	+
129	Camp Peary E × Paducah	7	34	0	41	17.1	+
133	Camp Peary L × Birch Tree 1	13	25	0	38	34.2	+
134	Camp Peary L × Birch Tree 2	12	22	0	34	35.3	+
136	Camp Peary L × Hot Springs	14	22	1	37	37.8	+
138	Camp Peary L × Paducah	0	24	0	24	0	
142	Friendship × Birch Tree 1	13	2	0	15	86.7	+
143	Friendship × Birch Tree 2	4	27	0	31	12.9	+
144	Friendship × Camp Peary E	37	4	0	41	90.3	+
146	Friendship × Hot Springs	37	0	0	37	100	+
148	Friendship × Paducah	6	33	0	39	15.4	+
152	Hopkinsville × Birch Tree 1	26	9	0	35	74.4	+
153	Hopkinsville × Birch Tree 2	7	12	2	21	33.3	+
154	Hopkinsville × Camp Peary E	28	10	0	38	73.8	+
156	Hopkinsville × Hot Springs	33	5	0	38	87.0	+
158	Hopkinsville × Paducah	0	39	0	39	0	
163	Hot Springs × Birch Tree 1	21	27	0	48	43.8	+
164	Hot Springs × Birch Tree 2	12	28	0	40	30.0	+
165	Hot Springs × Camp Peary E	16	16	0	32	50.0	+
168	Hot Springs × Paducah	2	35	0	37	5.4	+

TABLE 1. *Continued*

Culture no.	Cross	α·α	α·β	β·β	Total progeny	% α·α	Cytologically confirmed
172	Lake × Birch Tree 1	0	17	2	19	0	
173	Lake × Birch Tree 2	0	33	0	33	0	
174	Lake × Camp Peary E	2	24*	1	27	7.4	+
176	Lake × Hot Springs	10	18	1	29	34.5	+
177	Lake × Paducah	0	28	1	29	0	
181	Magnolia × Birch Tree 1	15	2	1	18	83.4	+
182	Magnolia × Birch Tree 2	15	21	0	36	41.7	+
183	Magnolia × Camp Peary E	28	1	0	29	96.6	+
184	Magnolia × Hot Springs	5	28*	0	33	15.1	+
186	Magnolia × Paducah	4	35	0	39	10.2	+
189	Paducah × Birch Tree 1	33	7	0	40	82.5	+
190	Paducah × Birch Tree 2	12	29	0	41	29.2	+
191	Paducah × Camp Peary E	33	3	0	36	91.6	+
193	Paducah × Hot Springs	26	15	1	42	62.0	+
200	Princeton × Birch Tree 1	0	14	0	14	0	
202	Princeton × Camp Peary E	19	17*	0	36	52.8	
204	Princeton × Hot Springs	18	18	1	37	48.6	
205	Princeton × Paducah	0	34	0	34	0	
208	Rich Mt. × Birch Tree 1	29	11	0	40	72.5	+
209	Rich Mt. × Birch Tree 2	4	29*	4	37	10.8	+
210	Rich Mt. × Camp Peary E	31	2	0	33	94.0	
212	Rich Mt. × Hot Springs	25	11	0	36	69.3	+
214	Rich Mt. × Paducah	0	35	3	38	0	
225	Warrenton × Birch Tree 1	32	9	0	41	78.0	
226	Warrenton × Birch Tree 2	14	21	0	35	40.0	
227	Warrenton × Camp Peary E	11	27	0	38	29.0	+
229	Warrenton × Hot Springs	35	2	0	37	94.8	
231	Warrenton × Paducah	5	48	0	53	9.4	+
217	Warwick A × Birch Tree 1	29	10	0	39	74.4	+
218	Warwick A × Birch Tree 2	10	29	0	39	25.6	
219	Warwick A × Camp Peary E	13	13	0	26	50.0	
221	Warwick A × Hot Springs	22	19	0	41	52.6	
223	Warwick A × Paducah	0	34	0	34	0	
235	Williamsburg × Birch Tree 1	0	42	0	42	0	
237	Williamsburg × Camp Peary E	21	21	0	42	50.0	+
239	Williamsburg × Hot Springs	22	14	0	36	66	+
240	Williamsburg × Paducah	0	31	0	31	0	

* The figures given include beta·alphas as follows:

Culture	beta·alphas
103	3
121	11
133	23
174	2
184	1
202	1
209	3

meiosis. No evidence was found of abnormal behavior during meiosis in any of the material.

The feature of the alpha·alpha combinations which is most striking and of central importance in further investigation is the fact that all of them are self-incompatible. No normal seeds were obtained from any of the controlled self-pollinations. In the second series (table 1), for instance, alpha·alpha combinations from thirty-three different cultures were selfed; in each case this involved usually three or four hand

TABLE 2

Frequency of alpha·alpha combinations in crosses of each race used as male parent

	% of α·α	
	Range	Average
1955 series		
Paducah	0–17.1%	6.1%
Birch Tree 1	0–86.7%	52.4%
Birch Tree 2	0–66.0%	25.8%
Camp Peary E	0–96.6%	52.6%
Hot Springs	15–100.0%	56.6%
1952 series		
Birch Tree 1	0–80%	53.7%
Birch Tree 2	0–41%	15.7%
Hot Springs	0–62%	40.6%

pollinations and from ten to twenty natural self-pollinations. None of them produced viable seed.

On the other hand, when an alpha·alpha is outcrossed, either as a male or female parent and either to normal races or to other alpha·alphas, abundant and normal seeds are produced. The pollen as well as the megaspores must, then, be viable and capable of producing normal gametophytes and gametes. Alpha·alpha combinations have been outcrossed successfully to the following races:

Bestwater 2	Friendship
Birch Tree 2	Hopkinsville
Camp Peary E	Hot Springs
Camp Peary L	Magnolia
Chapultepec	Paducah
Chicaginensis	Warwick A

The following alpha·alphas were intercrossed in all possible combinations:

α Hot Springs · α Birch Tree 2
α Camp Peary E · α Birch Tree 2
α Paducah · α Birch Tree 2
α Hot Springs · α Camp Peary E
α Paducah · α Camp Peary E
α Paducah · α Hot Springs

All crosses proved to be fertile.

Crosses between alpha·alphas which carry the same complexes but which were obtained from reciprocal crosses between races, are listed below. These, like the self-pollinations, were uniformly sterile.

α Birch Tree 1 · α Camp Peary E × α Camp Peary E · α Birch Tree 1
α Camp Peary E · α Birch Tree 1 × α Birch Tree 1 · α Camp Peary E

α Birch Tree 1 · α Hot Springs	× α Hot Springs · α Birch Tree 1
α Hot Springs · α Birch Tree 1	× α Birch Tree 1 · α Hot Springs
α Birch Tree 1 · α Paducah	× α Paducah · α Birch Tree 1
α Paducah · α Birch Tree 1	× α Birch Tree 1 · α Paducah
α Hot Springs · α Paducah	× α Paducah · α Hot Springs
α Paducah · α Hot Springs	× α Hot Springs · α Paducah
α Hot Springs · α Camp Peary E	× α Camp Peary E · α Hot Springs
α Camp Peary E · α Hot Springs	× α Hot Springs · α Camp Peary E
α Paducah · α Camp Peary E	× α Camp Peary E · α Paducah
α Camp Peary E · α Paducah	× α Paducah · α Camp Peary E

When an alpha·alpha is crossed to an alpha·beta of the same culture in either direction, seeds are always obtained. Such crosses are shown below:

α Paducah · β Hot Springs	× α Paducah · α Hot Springs
α Paducah · α Hot Springs	× α Paducah · β Hot Springs
α Paducah · β Birch Tree 1	× α Paducah · α Birch Tree 1
α Paducah · α Birch Tree 1	× α Paducah · β Birch Tree 1
α Magnolia · β Hot Springs	× α Magnolia · α Hot Springs
α Magnolia · α Hot Springs	× α Magnolia · β Hot Springs
α Magnolia · α Camp Peary E	× α Magnolia · β Camp Peary E
α Magnolia · α Birch Tree 2	× α Magnolia · β Birch Tree 2
α Magnolia · β Birch Tree 2	× α Magnolia · α Birch Tree 2
α Magnolia · β Birch Tree 1	× α Magnolia · α Birch Tree 1
α Magnolia · α Birch Tree 1	× α Magnolia · β Birch Tree 1
α Lake · β Camp Peary E	× α Lake · α Camp Peary E
α Lake · α Camp Peary E	× α Lake · β Camp Peary E
α Lake · β Hot Springs	× α Lake · α Hot Springs
α Lake · α Hot Springs	× α Lake · β Hot Springs
α Lake · α Hot Springs	× β Lake · α Hot Springs

A study was undertaken to determine whether the self-incompatibility might involve the failure of pollen tube growth. One to several styles from flowers of the following series of crosses were fixed twenty-four hours after pollination with alpha·alpha pollen:

Bestwater 2 × α Hot Springs · α Paducah
Chapultepec × α Hot Springs · α Paducah
Chicaginensis × α Hot Springs · α Paducah
Hopkinsville × α Hot Springs · α Paducah
Hot Springs × α Hot Springs · α Paducah
Magnolia × α Hot Springs · α Paducah
Paducah × α Hot Springs · α Paducah
Warwick A × α Hot Springs · α Paducah

Pollen tube growth extended to the base of the style in every case. Similar preparations, however, made in following self-pollination of alpha·alpha plants revealed an inhibition of pollen tube growth. Most pollen tubes either stopped growing shortly

after germination or did not proceed more than a few millimeters down the style. Occasionally, however, one or two tubes were observed at the base of the style. When the ovaries of such self-pollinated flowers were examined, it was noted that a few of the ovules had enlarged. The number of enlarged ovules varied from ovary to ovary; they ranged from one to as many as twenty-six, although usually there were fewer than a dozen. Gross dissection of such ovules revealed an embryo which at a later stage disintegrated to form a soupy liquid within a normal appearing seed coat. At the time the seeds would normally be mature, the capsule contained the dried undeveloped ovules and a few empty seed coats. The ovary usually enlarged slightly after pollination and persisted until the seeds would normally be mature. The self-incompatibility of the alpha·alpha combinations, therefore, can be attributed primarily to the failure of pollen tube growth. When a pollen tube occasionally does reach an embryo sac, the fertilization which occurs is unsuccessful because of the deterioration of the partially developed embryo. A more detailed study of the development of such an embryo has not yet been carried out.

Inbreeding of the seven-paired alpha·alpha combinations was originally attempted with the hope of obtaining an alethal, homozygous segregate which thus would be derived from a complex-heterozygote, but would show a return to the ancestral type of chromosome behavior. When self-pollination of the alpha·alphas proved unsuccessful, it was thought that a type approaching homozygosis might be obtained through a series of backcrosses. At the time, of course, the nature of the self-incompatibility was little understood. One of the alpha·alpha combinations, alpha Paducah·alpha Hot Springs, was backcrossed as the male parent to Hot Springs as the female parent. The progeny from this cross consisted entirely of structural homozygotes, since Hot Springs transmits only the alpha complex through the egg. Four plants from this progeny were crossed to Hot Springs, using the latter again as the female parent. All offspring from each of these crosses were structural homozygotes. A third backcross generation gave again only structural homozygotes. All offspring of the three backcross generations were self-incompatible.

Among the cultures grown during the 1955 season a number of beta·beta combinations also appeared. As will be noted in table 1, the frequency of this type of hybrid is small compared to that of the alpha·alpha. Since this material was at hand, however, MISS MARY SEAMAN began studies similar to those carried out with the alpha·alpha forms.

Only in one culture was the beta·beta a structural homozygote and thus strictly comparable. Interestingly enough, the plants of this combination were perfectly self-fertile. What the progeny will be like must await the coming growing season. Likewise, all other beta·betas, showing large or small circles at meiosis, proved to be self-fertile.

DISCUSSION

The most interesting feature of these results is the self-incompatibility of the alpha·alpha combinations. The consistency with which this behavior is shown by all alpha·alpha combinations so far produced points to its being a phenomenon of fundamental significance in the oenotheras of the biennis group 1. The pattern of self-

incompatibility strongly suggests that the alpha complexes possess an incompatibility allele system of the Nicotiana type. Such a system is already well known in *Oenothera organensis* (EMERSON 1938).

Conclusive evidence for the existence of an incompatibility allele system in the alpha complexes of the biennis group 1 will require an additional generation which will be grown during the coming summer. The results of a number of crosses which have already been carried out, however, are consistent with this hypothesis. Furthermore, predictions which on this basis can be made concerning the constitution of the next season's progenies, will serve as critical tests of the hypothesis.

The first line of evidence supporting the incompatibility allele hypothesis comes from the results of crosses between different alpha·alphas. If one assumes that the alpha complex of each race carries a distinctive incompatibility allele (designated by the letter *S*), then the crosses among different alpha·alphas should all be fertile, as the results indicate. Further, the compatibilities of the offspring from these crosses should be predictable. For example, assume that alpha Hot Springs carries S_1, alpha Birch Tree 2, S_2, and alpha Camp Peary E, S_3. Then, the cross: alpha Hot Springs (S_1)·alpha Birch Tree 2 (S_2) × alpha Camp Peary E (S_3)·alpha Birch Tree 2 (S_2) should produce both S_1S_3 and S_2S_3 progeny. The cross of S_1S_3 with alpha Hot Springs (S_1)·alpha Camp Peary E (S_3) should be incompatible, while S_1S_3 crossed with alpha Camp Peary E (S_3)·alpha Birch Tree 2 (S_2) should give seed. Likewise, the S_2S_3 offspring should give seed when crossed with alpha Hot Springs (S_1)·alpha Camp Peary E (S_3) but not with alpha Camp Peary E (S_3)·alpha Birch Tree 2 (S_2). This kind of analysis of the offspring from the crosses between different alpha·alphas should constitute, therefore, one test of the incompatibility allele hypothesis.

A second test is provided by the reciprocal crosses which have been made between alpha·alphas and alpha·betas of the same culture. On the basis of an *S* allele system, the results of these crosses are predictable. In the example which is given below, the race Paducah is involved; let us assign to the alpha Paducah complex the S_4 allele. Also, since our data show that beta·betas from the biennis 1 crosses are self-fertile, it must be assumed that *S* alleles are absent in the beta complexes. The corresponding locus in the beta complex can, then, be represented by the symbol, (+).

Parents: Paducah × Hot Springs

F_1: α Paducah (S_4) · α Hot Springs (S_1) × α Paducah (S_4) · β Hot Springs (+)
Progeny should consist only of alpha·betas.
α Paducah (S_4) · β Hot Springs (+) × α Paducah (S_4) · α Hot Springs (S_1)
Progeny should include alpha·alphas.

A third line of evidence comes from a series of crosses in which alpha·alphas were hybridized as female parents with races known to transmit their alpha complexes through the pollen. All of these crosses were fertile. According to the incompatibility allele hypothesis, if the female parent possesses an alpha complex in common with the race used as male parent, only the beta pollen should grow and the progeny will consist entirely of alpha·beta combinations. On the other hand, if the alpha·alpha used as the egg parent was derived from races other than that used as the male parent,

then alpha·alpha combinations should also appear among the progeny. Likewise, in crosses of races as female parents with synthesized alpha·betas, it should be possible to predict whether the progenies will contain only alpha·betas or both alpha·alpha and alpha·beta combinations. The validity of these predictions will be tested in the next growing season.

The incompatibility allele hypothesis provides an explanation of the results of backcrossing alpha Paducah·alpha Hot Springs to the Hot Springs parent for three generations. These crosses can be represented as follows:

F_1 * α Hot Springs (S_1) · β Hot Springs (+) × α Paducah (S_4) · α Hot Springs (S_1). Progeny all S_1S_4

F_2 α Hot Springs (S_1) · β Hot Springs (+) × S_1S_4. Progeny all S_1S_4

F_3 α Hot Springs (S_1) · β Hot Springs (+) × S_1S_4. Progeny all S_1S_4

The 1955 planting included three crosses in which an alpha·alpha (S_1S_4) from the second backcross generation described above was crossed as a female parent to the races Birch Tree 1, Hot Springs, and Paducah respectively. Since alpha Birch Tree 1 cannot carry the same S allele as either alpha Hot Springs or alpha Paducah, the S_5 allele can be assigned to alpha Birch Tree 1. The above crosses may then be represented with regard to S alleles as follows:

I (S_1S_4) × α Birch Tree 1 (S_5) · β Birch Tree 1 (+)
alpha·alphas (S_1S_5), or (S_4S_5)
alpha·betas (S_1+), or (S_4+)
Both alpha·alphas and alpha·betas appeared among the progeny.

II (S_1S_4) × α Hot Springs (S_1) · β Hot Springs (+)
Only alpha·betas are possible on the hypothesis and only alpha·betas appeared.

III (S_1S_4) × Paducah (S_4) · β Paducah (+)
Only alpha·betas are possible on the hypothesis and only alpha·betas appeared.

Each of the races used as male parent has been shown to transmit the alpha complex through the pollen, yet in these crosses alpha·alpha progeny occurred only in the Birch Tree 1 cross where, according to the hypothesis, one would assume that the incompatibility allele differs from those present in the female parent. Thus in crosses II and III the alpha pollen carrying either S_1 or S_4 apparently cannot grow in the style where these alleles are present.

It will be noted that in both the 1952 and 1955 series of hybrids, the crosses between Birch Tree 1 and Birch Tree 2 races have not produced any alpha·alpha combinations even though both races are known to transmit their alpha complexes through the pollen. Since these races were collected in the same locality, it is possible that their alpha complexes carry identical incompatibility alleles. If the incompatibility alleles in the alpha Birch Tree complexes are identical, then a cross between two alpha·alpha combinations which differ only in one possessing the alpha Birch

* Hot Springs transmits only the alpha complex through the egg.

Tree 1, the other the alpha Birch Tree 2 complex, should be sterile. Three such crosses, which test the identity of the incompatibility alleles in the alpha Birch Tree 1 and the alpha Birch Tree 2 complexes, are listed below; all produced seed.

α Camp Peary E · α Birch Tree 2 × α Camp Peary E · α Birch Tree 1
α Hopkinsville · α Birch Tree 1 × α Hopkinsville · α Birch Tree 2
α Birch Tree 2 · α Hot Springs × α Birch Tree 1 · α Hot Springs

The absence of alpha·alpha combinations among the progeny when these two races are crossed must not, therefore, result from the action of the incompatibility alleles. It should be noted that in the crosses between these races the number of seedlings which survive has been low. For example, in the cross of the 1955 series, Birch Tree 1 × Birch Tree 2, forty germinated seeds were planted; all of these were growing a week later, but after two additional weeks only nineteen survived. It may well be that some alpha·alphas were among the non-survivors. These results lead to the interpretation that alpha Birch Tree 1 and alpha Birch Tree 2 possess a common zygotic lethal. Thus zygotic lethals may be present in these complexes in addition to incompatibility alleles.

If an incompatibility allele system is present in the alpha biennis 1 complexes, it may have played a significant role in the origin and phylogeny of the biennis group 1. Earlier work (CLELAND, *et al.* 1950) has led to the belief that this group of complex-heterozygotes arose through the hybridization of previously isolated forms which had undergone cytogenetic differentiation. The alpha complexes presumably have been derived from an ancestral group of structural homozygotes not too greatly different from the present day grandifloras of the Southeast (STEINER 1952). On the other hand, this ancestral population must have acquired an incompatibility allele system which *Oenothera grandiflora* does not possess. The population from which the beta biennis 1 complexes have been derived, failed to develop such a self-incompatibility mechanism. When contact between the two populations occurred, hybrids were produced which possess ⊙ 14 chromosomes at meiosis because of the fact that the two populations had developed differences in chromosome end arrangement. Such forms, when self-pollinated, could not produce alpha·alpha segregates because of the incompatibility alleles. Thus the progeny presumably would consist of alpha·betas and beta·betas. To arrive at true-breeding heterozygotes such as the biennis group 1 oenotheras are, beta·beta offspring must not occur. RENNER (1940) has shown that in many forms a competition between megaspores exists, the genetic constitution of the megaspore determining which succeeds in developing into the embryo sac. Since the original hybrids between the progenitor groups here brought together involved two quite different genomes or complexes, it does not seem unlikely that megaspore competition occurred in the original hybrids, with the alpha complex succeeding. With only the alpha coming through the egg and only beta pollen functional, a true-breeding complex-heterozygote thus could have arisen simply by the act of hybridization between the two progenitor groups. Such a form would possess upon its inception a "balanced lethal mechanism", consisting of the incompatibility allele system on the one hand, and the megaspore competition on the other. "Balanced lethal mechanism" is, therefore, interpreted to include *any* mechanism which prevents the occurrence

of homozygotes of either type. This differs from the point of view of RENNER, who considers "lethal" in an absolute sense and makes a point of emphasizing that megaspore competition cannot be considered to fall into the category of a "lethal" (RENNER 1929, 1940).

Once such complex-heterozygotes became established, however, recessive lethal mutations which occurred in either of the complexes would be preserved because of the nature of the cytogenetic mechanism. After a period of time one would expect both the alpha and beta complexes of these forms to carry recessive lethal or deleterious genes which would reinforce the mechanism by which the complex homozygotes were originally eliminated from the progenies of the heterozygotes. The results of the crosses between the Birch Tree races provides evidence that the alpha complexes may carry zygotic lethals. The presence of deleterious recessives has been shown in alpha Beaufort, a complex occurring in one of the collections from the southeastern United States (STINSON and STEINER 1955). The fact that alpha·alphas can be obtained in so many of the crosses between different biennis 1 races does not deny the presence of zygotic lethals or deleterious genes. Since these complexes each stem from a different inbred line, during their history recessive lethal or deleterious factors which have arisen and have been preserved, are not likely to be identical in the different inbred lines. It should be emphasized, however, that the accumulation of recessive lethal or detrimental genes is a consequence of the cytogenetic mechanism characteristic of the true-breeding complex heterozygote; lethals of this type could, therefore, not have factored in the original establishment of the true-breeding complex heterozygote.

It is interesting to note that the self-incompatibility which is described here as a characteristic of the alpha·alpha combinations was reported by ATKINSON (1917) in a hybrid obtained by crossing *Oenothera nutans* Atkinson and Bartlett and *Oenothera pycnocarpa* Atkinson and Bartlett. From ATKINSON's description he unquestionably had encountered the same behavior described above. It is difficult to say from the descriptions of the two species whether they belong to the biennis 1 group. CLELAND (personal communication) has stated that collections which came to him under the names *Oe. nutans* and *Oe. pycnocarpa*, upon cytogenetic analysis turned out to belong to the biennis group 2. Thus there is some indication that the self-incompatibility mechanism may not be restricted to only one phylogenetic group.

Another interesting feature of some of the crosses made in this study which requires further analysis has been called to my attention by DR. STERLING EMERSON. When races which are known to transmit the alpha complex through the pollen are crossed reciprocally, one generally obtains a much larger percentage of alpha·alpha combinations in one direction than in the other. A group of such crosses is listed in table 3. Where data are available for two seasons, it is apparent that wide variation in the percentages of the alpha·alphas may occur. Only in one case, namely, the crosses between the Birch Tree 2 and Hot Springs, is the percentage of alpha·alpha combinations roughly equal in reciprocal crosses. Thus failure to obtain alpha·alphas in one direction cannot be attributed to zygotic lethals, but must result from some other mechanism which may also account for the variation in the frequency of transmission of the alpha through the pollen in different crosses.

TABLE 3

Alpha·alpha combinations produced in reciprocal crosses between races of the biennis group 1 which transmit the alpha complex through the pollen

Cross	1952		1955	
	Ratio	Percent	Ratio	Percent
Birch Tree 1 × Birch Tree 2	0/43	0	0/21	0
Birch Tree 2 × Birch Tree 1	0/36	0	—	—
Birch Tree 1 × Hot Springs	1/35	2.8	7/31	22.0
Hot Springs × Birch Tree 1	38/57	67.0	21/48	43.8
Birch Tree 2 × Hot Springs	0/57	0	6/22	27.2
Hot Springs × Birch Tree 2	23/56	41.0	12/40	30.0
Birch Tree 1 × Paducah	1/15*	6.6	2/39	5.1
Paducah × Birch Tree 1	4/5	80.0	33/40	82.5
Birch Tree 2 × Paducah			2/37	5.4
Paducah × Birch Tree 2	1/63	1.5	12/41	29.2
Birch Tree 1 × Camp Peary E			1/20	5.0
Camp Peary E × Birch Tree 1			14/35	40.0
Birch Tree 2 × Camp Peary E			0/15	0
Camp Peary E × Birch Tree 2	2/30†	7	25/38	66.0
Hot Springs × Camp Peary E			16/32	50.0
Camp Peary E × Hot Springs			30/37	81.1
Hot Springs × Paducah			2/37	5.4
Paducah × Hot Springs	42/69	61.0	26/42	62.0
Camp Peary E × Paducah			7/41	17.1
Paducah × Camp Peary E			33/36	91.6

* Data from CLELAND.
† Data from STINSON.

It is fully recognized that the proposed explanation of the above data still remains essentially a working hypothesis. Many of the predictions which have been made will be tested with the coming season's progenies. Studies are also under way to determine why a few pollen tubes function in fertilizations when the alpha·alphas are selfed, and what the significance of the degenerating embryos may be. Other groups are being surveyed for the presence of self-incompatibility among their hybrids. It is likely that EMERSON's technique (EMERSON 1938) for testing compatibilities by determining pollen tube growth may be utilized here. With further work a future report should allow a more complete interpretation of these results in the light of our present understanding of the entire Oenothera picture.

SUMMARY

1. Oenotheras belonging to the biennis 1 phylogenetic grouping are true-breeding heterozygotes which are composed of two genomes, the alpha and beta complexes, each of which possesses a distinctive arrangement of chromosome ends.

2. In crosses between many of the biennis group 1 races it is possible to obtain viable hybrids which carry the alpha complex from each parent.

3. Such alpha·alpha combinations are self-incompatible, but fertile in outcrosses.

4. Beta·beta combinations which also occur among some of the hybrid progeny at a low frequency are self-fertile.

5. The hypothesis is proposed that the alpha biennis 1 complexes carry an incompatibility allele system of the Nicotiana type, while such a system is absent in the beta biennis 1 complexes.

6. If an incompatibility mechanism is operative and if megaspore competition occurs, it is possible to explain the origin of the true-breeding complex-heterozygote simply by the act of hybridization. The self-incompatibility system together with megaspore competition constitutes a balanced lethal mechanism.

7. Recessive lethal or detrimental genes which may exist in the biennis 1 complexes appear to have arisen after complex-heterozygotes occurred; they played no role in the original establishment of true-breeding structural heterozygotes.

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CHROMOSOMAL ADAPTIVE POLYMORPHISM IN DROSOPHILA PERSIMILIS. I. LIFE CYCLE COMPONENTS UNDER NEAR OPTIMAL CONDITIONS[1]

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Received January 14, 1956

AT least three principal facts concerning the adaptive functions of chromosomal variants (inversions) in Drosophila populations have been established notably by DOBZHANSKY (1947, 1955), DOBZHANSKY and WALLACE (1953), DOBZHANSKY and LEVENE (1951), DOBZHANSKY and PAVLOVSKY (1953), and WALLACE (1953): 1) Natural selection may build up particular genic complexes in a chromosomal variant adapting homokaryotypes for specific environments; 2) natural selection may "co-adapt" heterozygous genic complexes by acting on heterokaryotypes involving two different chromosomal arrangements from one interbreeding population so that they display greater fitness than homokaryotypes; and 3) heterokaryotypes which have been coadapted to permit survival of balanced chromosomal polymorphism often are heterotic and homeostatic for traits which contribute to their adaptedness, that is, individuals show heterosis and more constant phenotype in developmental and physiological processes throughout a wider range of environments. This last fact has been extended in LERNER's (1954) concept of the "buffering" of developmental processes (or "canalization", WADDINGTON 1942) indicated by a lowered environmental variance among heterozygotes which display heterosis.

In order to establish the nature of co-adaptation between gene complexes of two different chromosomal arrangements three procedures have been employed customarily: 1) sampling of larvae from population cages containing two or more chromosomal variants to determine relative frequencies of the variants with time, 2) counting ratios of normal to dominant marker flies in crosses of wild-dominant marker heterozygotes to determine relative viabilities and developmental rates of "wild" and dominant phenotypes, and 3) measuring various physiological components of the fly life cycle to determine the expression of the genic complexes included by the inversion.

This last method has one major advantage over the dominant marker cross technique, namely progeny of wild flies are tested without crossing to a laboratory strain, and consequently the genic milieu has a history determined by evolutionary forces and not unrelated to the genic complex included within the chromosomal variant. On the other hand, a comparison between results of these various methods employed on one pair of gene arrangements would promise far more information than the results of only one method by itself.

It was shown by the senior author (SPIESS 1950) that *Drosophila persimilis* indi-

[1] Work reported in this article has been carried out in preliminary analysis under a grant-in-aid from the American Academy of Arts and Sciences, Boston, Massachusetts and has been completed under contract No. AT-(30-1)-1775 with the U. S. Atomic Energy Commission.

viduals possessing either the "Whitney" (*WT*) or "Klamath" (*KL*) inversion or both from a locality in the San Joaquin Valley of California (Jacksonville, elevation 800 ft.) are not measurably different in adaptive values at 25°C in population cages, but at 16°C heterokaryotype flies (*WT/KL*) displayed net superiority and equilibrium was established at 51% *WT*. Fecundity of females raised under crowded conditions showed that *WT/WT* flies were superior to *KL/KL* and that *WT/KL* were superior to both. A maternal effect on this heterosis was also discovered (SPIESS, KETCHEL, and KINNE 1952).

In the summer of 1953 the senior author and his family collected flies from several high elevation localities in the Yosemite Region. The locality which was chosen for detailed study, White Wolf Lodge Pond, elevation 8000 ft., is in lodgepole pine forest (*Pinus contorta*) with very little underbrush, sparse soil, and extensive bedrock outcroppings. Frequencies of karyotypes have been calculated as follows (SPIESS 1953) *WT* 87%, *KL* 7%, *MD* 3%, *ST* 2% and *SE* 1%. These frequencies agree with DOBZHANSKY's data (1948) for localities near 8000 ft. Indications are that *KL* has increased by about 5% at the expense of *ST* over the intervening five years. Collections of DOBZHANSKY in 1954 (in press) corroborate this decrease in Standard and increase in Klamath.

With fresh stocks of flies the solution of three main problems has been undertaken: 1) the relative adaptive values of gene arrangements from this locality computed by population cage technique for comparison with the same arrangements from other localities; 2) the components of the fly's life cycle displaying phenotypic differences between karyotypes: are differences evident in particular components of the life cycle or do differences between karyotypes display a general overall uniformity throughout the life cycle? 3) a possible demonstration of heterosis in particular stages of the life cycle and finding evidence of lowered environmental variance to substantiate LERNER's hypothesis of homeostasis in the heterozygote, a greater ability "to stay within the norms of canalized development".

POPULATION CAGE DATA

WT and *KL* flies were introduced into plastic population cages, and after many generations relative adaptive values of three karyotypes were calculated. (For details of culture technique and computation of adaptive values, see SPIESS, in press). Two simultaneously run cages indicate an equilibrium at 70–75% *WT* and 25–30% *KL* at 16°C (relative adaptive values: 0.28 *KL/KL*: 1.00 *WT/KL*: 0.75 *WT/WT*).

MATERIALS AND METHODS

A knowledge that two chromosomal arrangements differ in their adaptive responses to the conditions of a population cage raises the question stated above (# 2, # 3), and in attempting to trace those developmental and physiological processes controlled by a particular karyotype it seems necessary to measure life cycle components under near-optimal conditions first and later to simulate population cage conditions by increasing population density and by introducing more than one karyotype into the culture. All experiments reported here employed cultures of low population density in comparison with population cage food dishes, only one karyo-

type was raised in any single culture, and results can be referred to as "standard" for future reference.

Four strains of *WT* and four of *KL* were chosen at random from an original sample of nearly fifty wild strains, each strain consisting of the descendants of a single wild female caught at White Wolf Pond, elevation 8000 ft. All *KL* strains were derived from *WT/KL* wild females by sib mating for about five generations in order to produce homokaryotype *KL*. *WT* strains were subjected to only two or three initial generations of sib mating. Flies to be tested were usually F_1 individuals from strain crosses. Some tests were performed on original strain flies for the sake of comparison with strain cross F_1's. Four strain crosses were made for each karyotype: $WT_1 \times WT_2$, *2* × *3*, *3* × *4*, *4* × *1*: Series A–D; $KL_1 \times KL_2$, etc.: Series E–H; $WT_1 \times KL_1$, etc.: Series I–L; and $KL_1 \times WT_1$ etc.: Series M–P.

This plan of mating was chosen to represent a population of karyotypes approximating a "wild" genotype and at the same time provide a basis for calculation of uniformity between genotypic combinations resulting from each cross. Does the hypothetical gene complex organized as a supra-genic unit with a particular adaptive value become expressed unalterably in all combinations of that unit? Does the heterotic genotype demonstrate lowered environmental variance also? Others (Moos 1955; Heuts 1948; Wallace 1948; and Levine 1952) who have studied life cycle components of *D. pseudoobscura* possessing certain gene arrangements either do not record variance between strain crosses or they synthesize an F_1 population by crossing several strains at random in an experimental cage so that variance between crosses and within crosses cannot be distinguished.

Adult females to be tested for fecundity were raised as follows: stock parents (strain #1 ♀♀ × strain #2 ♂♂) were mated in several replicate bottles about six to ten pairs per bottle and provided with yeasted food on plastic spoons. Spoons on which F_1 eggs had been laid were withdrawn, counted for eggs, and inserted into the food medium of ordinary culture bottles; no more than 100 eggs were put into any culture. Adult females from at least three and no more than five of these culture bottles were isolated and tested for egg-laying capacity.

For tests of egg hatchability, survival of larvae, and rate of development, matings were made between strains in the same manner. Eggs collected from the spoons were washed first in 70% ethyl alcohol, then in sterile water to cut down contamination, and finally planted on a nutrient agar surface in 9 cm petri dishes. Eggs were planted evenly in numbers of 50–100 per petri dish; aggregation of eggs into crowded groups lowers the survival percentage considerably (Grucci 1954).

Cream of wheat-molasses food medium inoculated with Fleischmann's Bakers Yeast was used for cultures of strains. The test medium for developmental stages was devised from nutrient agar of near transparency so that larvae could be identified at all stages (Schuellein 1954). All experiments were carried out at 15°C.

OBSERVATIONS

Egg-laying capacity

Females to be tested were mated to sibling males in glass vials one pair per vial, and food to which lampblack had been added was introduced on plastic spoons. Every two days spoons were changed and eggs counted. Records were kept only for

TABLE 1

Fecundity of females in average number of eggs per day in terms of ten day periods and overall average for the first 30 days of egg-laying. N = *no.* ♀♀. *C. V. = Coefficient of variability within series*

	Karyotype	Mean (Eggs/day)	*N*	σ^2 (Between series' means)	$\bar{\sigma}^2$ (Within series)	C.V.
1–10 days	WT_1/WT_2	20.28	78	2.89	14.66	0.182
	KL_1/KL_2	18.56	72	0.87	14.74	0.207
	WT_1/KL_1	20.34	92	1.96	11.60	0.168
	KL_1/WT_1	18.56	79	14.34	10.48	0.174
11–20 days	WT_1/WT_2	17.58	77	15.29	12.02	
	KL_1/KL_2	17.61	59	0.59	11.89	
	WT_1/KL_1	20.25	82	4.33	14.81	
	KL_1/WT_1	19.28	70	4.14	14.70	
21–30 days	WT_1/WT_2	17.37	61	2.96	10.73	
	KL_1/KL_2	18.29	38	2.32	14.56	
	WT_1/KL_1	19.68	71	1.27	13.42	
	KL_1/WT_1	19.39	60	7.03	20.65	
Average for 30 day period	WT_1/WT_2	18.46	61	1.98	6.07	0.133
	KL_1/KL_2	18.40	38	0.46	5.48	0.127
	WT_1/KL_1	20.08	71	0.51	5.72	0.119
	KL_1/WT_1	19.23	60	7.89	7.28	0.140

the first thirty days; in previous work it has been shown that *D. persimilis*'s average rate of production remains quite constant for more than sixty days and that any differences which exist between karyotype performances may be recognized from the start, although daily production may be very uneven (SPIESS, KETCHEL, and KINNE 1952). It has also been shown that the particular culture conditions in which females were raised had no measurable bearing upon the maternal effect in heterokaryotypes: as long as conditions of crowding among developing larvae were of the same magnitude it made no difference whether flies came from several population cage food cups or from culture bottles. Every attempt was made in the present experiments to keep culture and testing conditions uniform.

Data are given in eggs per day per female in ten day intervals and the average of the thirty day period in table 1. If the original strains had been highly inbred and were reasonably homozygous, we could state that most of the between series means was genetic and most of the variance within series (replicate crosses of strains) was environmental, assuming sampling error to be a component of variance equally distributed to each. The original strains have not been consistently inbred, however; replications of strain crosses will not give genetically uniform results, nevertheless it is likely that over the time these strains have been maintained in the laboratory some homozygosity must be attained because in chromosome analysis for the first four or five generations sib mating had been carried out, but since that time the strains have been mass mated. These variances are relative to the amount of genetic uniformity within strains but the component between means indicates a measure of

TABLE 2

Percent hatchability of eggs, i.e. number of first instar larvae per 100 eggs

Karyotype	m(%)	No. petri dishes	σ^2 (Between series means)	$\bar{s}^2$ (Within series)	C.V.
WT_1/WT_2	86.4	42	11.76	45.87	0.078
KL_1/KL_2	87.3	30	2.75	66.48	0.093
WT_1/KL_1	94.0	26	7.76	9.11	0.034
KL_1/WT_1 (omitting M series)	90.4	24	19.77	20.47	0.053
WT_1/WT_1	72.6	19	641.27	67.28	0.105
KL_1/KL_1	78.5	17	190.7	97.86	0.126
WT_1/WT_2 (transformed to $\sin^2\theta$)	68.93	—	9.60	36.87	0.088
KL_1/KL_2 (transformed to $\sin^2\theta$)	70.03	—	1.94	48.61	0.099
WT_1/KL_1 (transformed to $\sin^2\theta$)	76.90	—	17.42	15.38	0.051

genetic differences between strain crosses and therefore the genetic uniformity within any karyotype while that component within series indicates how the karyotype as a unit performs in environments which differ by relatively minor factors.

Among the data for ten day intervals no differences between means are statistically significant. Most strain crosses give uniform results (σ^2 between means) except KL/WT in the initial ten days and WT/WT in the second ten days. KL/KL is exceedingly uniform throughout.

The 30-day average has consistently lower variance both within and between series. This fact suggests the 30-day average to be a more reliable measure of fecundity than the short 10-day periods.

Heterosis for WT/KL is not marked if indeed present at all. In the 30-day average WT/KL differs from WT/WT by 1.62 eggs per day ($t = 2.05$, $d.f. = 6$, $P = 0.08$) and from KL/KL by 1.68 eggs per day ($t = 3.41$, $d.f. = 6$, $P = 0.014$). Of course the latter is much more uniform genetically ($\sigma^2 = 0.46$) than the former ($\sigma^2 = 1.98$). Homeostasis is also not apparent to any degree.

Coefficients of variability have been calculated for the first period of ten days and for the 30-day average. LERNER (1954) has followed the method of GUSTAFFSON (1946) in calculating the coefficient of variability for numerous experiments in which heterozygotes and homozygotes could be compared, but he states "... when the means and the variances are correlated, the coefficient of variation is not an entirely independent criterion". The coefficient of variability does not seem to be reliable as a measure of lowered variability unless the mean increases with an accompanying decrease in variance. It happens that the data in table 1 will give a low C.V. for heterokaryotype (WT/KL) but none of the C.V.'s are significantly different from any other because variances and means are so close.

A maternal effect in the heterokaryotype is not obvious from the data in table 1. Table 3 gives a breakdown of 30-day average data contributed by each series cross to show reciprocal differences in fecundity among heterokaryotype females. Series I and M, J and N, and so forth are reciprocals. Note three crosses are lower and one higher when KL is the mother. Two differences are highly significant and one is nearly significant. Apparently whatever uniformity of phenotype might be gained by heterozygosity in the heterokaryotype using a WT mother is lost by using a KL

TABLE 3

Series averages demonstrating reciprocal differences in the heterokaryotype for 30 day average fecundity and for hatchability of eggs

		Mean (Eggs/day)	N	σ_m	Mean (% hatchability)	N	σ_m
F_1	$WT_1 \times WT_2$						
	A	19.44	19	0.514	86.5	9	2.12
	B	17.00	20	0.635	83.6	8	2.55
	C	17.54	7	0.973	91.2	13	1.76
	D	19.87	15	0.552	84.3	12	1.87
F_1	$KL_1 \times KL_2$						
	E	18.26	14	0.581	87.4	9	2.85
	F	18.31	11	0.602	86.9	7	2.04
	G	19.34	7	0.719	85.5	7	3.78
	H	17.71	6	1.27	89.5	7	2.21
F_1	WT♀♀ × KL♂♂						
	I	20.19	16	0.314	91.7	4	0.687
	J	20.58	21	0.457	95.8	7	1.61
	K	20.50	19	0.553	97.0	6	0.808
	L	19.04	15	0.814	91.6	9	0.934
F_1	KL♀♀ × WT♂♂						
	M	18.33	11	1.05	78.5	7	2.16
	N	23.30	14	0.539	89.7	7	1.27
	O	16.84	18	0.601	95.2	8	0.878
	P	18.47	17	0.620	86.4	9	2.21
			t	P		t	P
		I–M	1.87	0.062	I–M	4.45	0.0017
		J–N	3.82	0.0006	J–N	2.75	0.016
		K–O	4.49	0.0001	K–O	1.46	0.16
		L–P	0.528	0.63	L–P	2.17	0.043

mother. Specific combining ability factors producing greater or less compatibility between egg cytoplasmic determiners and the father's genotype might be concerned in the causation of this maternal effect. Why this large variance should be evidenced by *KL/WT* flies and not by *KL/KL* is hard to explain on any other basis.

It is of interest to compare fecundity of these flies with those from Jacksonville raised in substantially the same conditions (SPIESS 1954). *WT/WT* females from the near sea level locality average 20 eggs per day, which is not significantly different from the data included here for this karyotype. Evidently *D. persimilis* from 8000 ft. and from 800 ft. are alike in fecundity under these laboratory conditions.

Hatchability and survival

As eggs hatched, a daily check on numbers of larvae was made with a tabulation grid designed to fit the petri dish. When larvae reached the third instar, a piece of

TABLE 4

Percent eclosion of adults per 100 eggs

Karyotype	$m(\%)$	No. cultures	s^2 (Between series means)	$\bar{s}^2$ (Within series)	C.V.
WT_1/WT_2	84.6	18	4.77	17.37	0.049
KL_1/KL_2	80.8	17	14.86	13.95	0.046
WT_1/KL_1	89.4	20	2.56	13.59	0.041
KL_1/WT_1 (omitting M)	88.6	14	1.12	12.25	0.040
WT_1/WT_1	60.1	17	657.0	14.10	0.062
KL_1/KL_1	70.4	14	353.6	71.96	0.120

paper toweling was placed on the agar surface. Most larvae pupated upon the towelling, and stray pupae were transferred to it.

Table 2 shows relative hatchability of eggs. *WT/WT* and *KL/KL* eggs hatch about equally when their heterozygosity is increased by strain-crossing. Individual strains of both karyotypes average much lower but are extremely heterogeneous: the individual *WT* strains averaged 52.4, 94.5, 98.0, and 45.6% hatchability while the *KL* strains averaged 70.5, 63.1, 90.9, and 89.4%. It is apparent no true mean can be calculated from these data for these individual strains, and at first glance the F_1 results in table 3 might appear to indicate the presence of dominant hatchability alleles in certain strains. That such an hypothesis is erroneous becomes clear when the F_1 series crosses are known: see table 4. Series A is the F_1 of 52.4 × 45.6 above, B is 52.4 × 94.5, etc., series E is the F_1 of 70.5 × 63.1 above, F is 63.1 × 89.4, etc. Variances within individual strains are of the same order of magnitude as those of their F_1's but are slightly larger in the single strains. The great increase in uniformity of means and the decrease in the environmental component of variance in homokaryotype F_1's indicate a homeostatic heterozygosity among both *WT* and *KL* strain cross progeny.

Heterokaryotype *WT/KL* displays a uniformly higher hatchability percent than any other karyotype with a markedly low average variance within series (difference between *WT/WT* and *WT/KL* is 7.6%, $t = 3.44$, $d.f. = 6$, $P = 0.013$; difference between *KL/KL* and *WT/KL* is 6.7%, $t = 3.53$, $d.f. = 6$, $P = 0.012$). There is evidently greater viability and "buffering" to be obtained by heterozygosity for loci in the heterokaryotype than for loci in the homokaryotype. Evidently heterokaryotypes are superior to homokaryotypes. This must also imply a greater amount of heterozygosity in the heterokaryotype since the two inversions are nearly independent systems.

Using percentages close to 100% for calculation of variance may be open to criticism; therefore the data used in calculating the top three rows of table 2 were transformed to $\sin^2 \theta$. Variance within series for *WT/KL* is still smallest: $F = 2.40$ comparing WT_1/WT_2 to WT_1/KL_1 ($d.f. = 38$ and 22, respectively) gives a probability of 0.02. Therefore it appears well-founded that the environmental component of variance in the heterokaryotype from *WT* mothers is significantly less than in either homokaryotype.

The reciprocal cross (*KL/WT*) however does not produce either such uniformity

or high viability. Table 3 gives a breakdown of individual series crosses to show the maternal effect in the heterokaryotype. The "M" series is especially striking in low viability compared with its reciprocal "I"; the original strains of *WT* and *KL* average 52.4% and 70.5%, respectively. Series M data have been omitted from the mean calculations of tables 2 and 4 because of an exceptionally low hatchability value. This series was repeated seven times and gave consistently low egg viability results. Presumably there must be specific incompatability between the two parent genotypes when *KL* is the mother which is not generally found among all the other crosses. Inclusion of M data would give an exceptionally low average for the *KL/WT* karyotype, which would be misleading in the reported average for that karyotype. Reciprocal series J–N and L–P are also significantly different though not so strikingly. In all cases *KL* mothers seem to lower the average viability of their offspring.

Unfortunately, observation of late larval stages in petri dishes is made difficult by the vigorous wandering of the creatures. Third instar larvae often leave the petri dish so that survival percentage can no longer be calculated although data for rate of development can still be obtained for those which do not escape. The problem was partially solved by paraffining the edge of the dish so that the cover would fit more snugly. Data given in table 4, however, were obtained completely on flies raised in half-pint bottles containing the same nutrient agar medium which had been used in the hatchability and rate of development experiments. These data serve as a check on percentages of survival obtained from petri dishes and are not significantly different from them.

Percent eclosion is a partial function of percent hatchability. The ratio of % eclosed/% hatched subtracted from 100% is a measure of larval and pupal mortality. The average mortality of *KL/KL* (7.5%) is the greatest for any of the karyotypes, none of which are significantly different. The high variance between means for *KL/KL* eclosion results from the mortality percent of one series cross, the other three average much more closely at a higher figure: E = 1.9%, F = 12.1%, G = 6.1%, H = 9.5%. The mortality of the E series is nearly equal to that of *WT/WT* 2.1%.

Superior eclosion values among the heterokaryotypes (omitting M) stems from their superior hatchability. Mortality is 4.8% for *WT/KL* and 2.0% for *KL/WT* (omitting M). Mortality for the M series is 7.0% and is within the range of the other heterokaryotype series. The M series had the very lowest egg viability but is not significantly low in postembryonic viability. Therefore it can be seen that differences initiated by viability of eggs are not radically altered by larval and pupal mortality.

The maternal effect at hatching is less marked at eclosion. There are no significant differences between reciprocals for eclosion values except I–M, but this difference was initiated at hatching. Relative survival of larvae and pupae do not display maternal effect.

While the original strains of *WT* and *KL* average the same mortality of larvae and pupae, inspection of individual strain data demonstrates the diversity of the strains and in some cases the lowered viability of nearly all with respect to their F_1's. *WT* strains showed 14.7%, 48.5%, 31.3%, and 0% while *KL* strains showed 16.1%, 20.8%, 1.9% and 6.8% mortality of larvae and pupae. Apparently heterosis and

TABLE 5

Percent survival of adult females from start of egg-laying experiment to the end of 30 days

Karyotype	*N*	% Survivors		
		10 days	20 days	30 days
WT_1/WT_2	92	88.0	82.6	69.6
KL_1/KL_2	92	87.0	71.8	58.7
WT_1/KL_1	111	90.9	82.7	71.8
KL_1/WT_1	106	86.8	70.8	59.5

uniformity are both gained by crossing strains: among *WT* crosses the 14.7% × 48.5% gave 2.2% mortality while among *KL* crosses the 16.1% × 20.8% gave 2.0% mortality.

It is reasonably clear that karyotype differences are mostly determined by egg hatchability where "buffering" is quite marked in the heterokaryotype, and that all karyotypes survive about equally in larva and pupa. This result agrees with figures obtained on pupal survival among flies from Jacksonville, California (SPIESS, YANKOPOULOS, and HUTCHINSON 1952).

Percent of adult female survival was calculated for those flies which were used in the egg-laying experiment (see table 5). *WT/KL* and *WT/WT* survive about equally well, while *KL/WT* and *KL/KL* are decidedly less viable. This result differs somewhat from results obtained on flies from low elevation (SPIESS, KETCHEL, and KINNE 1952), where no maternal effect on survival could be demonstrated, and both *WT* and *KL* flies were less viable than *WT/KL*. However the two experiments are not strictly comparable because the Jacksonville flies were cultured under crowded conditions, a factor which might have considerable bearing upon the viability of survivors.

Rate of development

Table 6 shows average number of days in each of the post-embryonic stages of development and the total time from egg to adult.

In both first and second instars *WT/WT* is faster than *KL/KL* with *WT/KL* intermediate. No maternal effect on the heterokaryotype can be detected. There is no heterosis in the heterokaryotype and no substantial lowering of average variance within series. Apparently the *WT/WT* gene complex develops faster than the other karyotypes and has the lowest environmental component of variance. Original strain flies take as long a time as *KL/KL* to go through these instars and in general display a larger average variance within series, showing that the environment has more influence upon them than upon their F_1's.

It is in the third instar, the most prominent of the three larval stages, that marked heterosis for *WT/KL* is expressed. Also *WT/WT* is still faster than *KL/KL*. Most interesting are the low variances both within series and between series means for the fastest karyotypes. F_1 *WT/WT* has much lower variances than single strains of *WT* showing not only genetic uniformity but homeostasis brought about by the heterozygosity increase. This effect is no evidenced by the F_1 *KL/KL* which takes longest

TABLE 6

Rate of development in post-embryonic stages (three larval instars, pupa, and total time from hatching to eclosion)

Karyotype	Life cycle period	Mean days	No. cultures	σ^2 (Between series means)	Av. σ^2 (Within series)
WT_1/WT_2	Larval Instar 1	2.9	32	0.515	0.097
	2	2.5	32	0.084	0.092
	3	11.0	32	0.063	0.512
	Pupa	12.9	32	0.153	0.151
	Total	29.3	32	0.396	2.570
KL_1/KL_2	Larval Instar 1	4.1	25	0.140	0.140
	2	3.4	23	0.022	0.107
	3	13.1	23	2.005	1.666
	Pupa	14.4	23	0.327	0.673
	Total	35.0	23	5.522	3.607
WT_1/KL_1	Larval Instar 1	3.4	25	0.388	0.170
	2	2.8	24	0.075	0.066
	3	8.2	24	0.413	0.565
	Pupa	12.6	23	1.732	0.239
	Total	27.0	23	3.978	0.442
KL_1/WT_1	Larval Instar 1	3.5	25	0.083	0.165
	2	2.7	25	0.038	0.119
	3	9.8	25	0.261	0.588
	Pupa	13.2	25	0.164	1.709
	Total	28.2	25	0.730	1.884
WT_1/WT_1	Larval Instar 1	4.6	17	0.915	0.309
	2	3.5	17	0.620	0.255
	3	10.5	16	3.352	1.668
	Pupa	14.7	16	0.879	0.504
	Total	33.3	16	19.538	5.762
KL_1/KL_1	Larval Instar 1	4.3	17	0.143	0.498
	2	3.3	17	0.067	0.108
	3	12.1	17	0.876	1.121
	Pupa	14.5	17	0.008	0.409
	Total	34.2	17	5.489	4.382

to develop and no lowering of variance with respect to its parental strains. These facts would tend to indicate that heterozygosity in the residual genome has little heterotic effect on rate of development, since *KL* F_1's and *WT* F_1's must have equal heterozygosity except on chromosome III.

WT/WT pupae develop faster than *KL/KL* by about one and one half days while *WT/KL* are equal to *WT/WT*. In this period *WT* seems to have a dominant effect in the heterokaryotype, and its low environmental variance component is repeated in pupae from *WT* mothers. There is no significant maternal effect in rate of pupal development of heterokaryotypes although variance within series is relatively large in pupae from *KL* mothers. Both variance components are greater for *KL/KL* F_1's

than their parental strains, while the reverse is true for *WT/WT*. This pattern is similar to that in the third instar with regard to the relative effect of residual genome heterozygosity upon increase of developmental rate. Apparently the *WT* chromosome must differ from *KL* in possession of heterotic loci governing rate of post-embryonic development, and loci on non-homologous chromosomes do not directly affect this trait in any degree when heterozygous.

The total days from hatching to eclosion can serve as a measure of net rate of development for these karyotypes. Like the percent eclosion figures given above, this measure represents several parameters, but many interesting net effects bearing on the behavior of these karyotypes are obvious. *WT/WT* is faster than *KL/KL* by about six days and shows greater genetic and environmental uniformity. *WT/KL* has the most rapid rate of all and the lowest environmental component of variance. There is no marked reciprocal effect. Heterosis and lowered variances in all F_1's except *KL/KL* show that the final effect is due to increased heterozygosity for loci on *WT* vs. *KL* arrangements of chromosome III in contrast to the effect observed in percent survival which can be accounted for by heterozygosity throughout the genome.

More evidence that the difference in rate of development between *WT* and *KL* is little affected directly by the residual genome is given in some data heretofore unpublished: the orange recessive marker and the orange-Blade-Scute-purple marker stock for chromosome III (see DOBZHANSKY and SPASSKY 1953) were backcrossed for five generations to a strain of *WT* from White Wolf Pond. Then by following the technique outlined in the above reference *WT* and *KL* chromosomes were made homozygous on a common residual genome derived from one wild strain. Normal flies should average 33.3% of the total when counted with dominant marker (Blade-Scute) in the analysis ratio. Exactly the same strain numbers, now outcrossed to the marker stocks, were crossed to simulate series crosses comparable to those studied above. Chromosome III was genetically comparable with that of the wild strain crosses tested in the above experiments, but the residual genome was far more uniform since it was derived from one wild strain. Results in terms of percent normals (non-Blade) in five day intervals of counting are given below:

		1st 5 days	*N*	2nd 5 days	*N*	3rd 5 days		Average	*N*
$\frac{or\ Bl\ Sc\ pr}{WT_1} \times \frac{or\ Bl\ Sc\ Pr}{WT_2}$	–	35.8%	830	35.5%	265	23.9%	167	34.6%	1262
$\frac{or\ Bl\ Sc\ pr}{KL_1} \times \frac{or\ Bl\ Sc\ pr}{KL_2}$	–	33.3%	603	30.4%	171	33.6%	152	32.8%	926

Even on a comparatively uniform genetic background the WT arrangement controls faster development than *KL*, but total survival is not significantly greater for one homokaryotype than the other as can be seen from the average percent.

DISCUSSION

Net adaptive value

Granted that there are other factors in the fly life cycle which have yet to be tested, it is of interest to compare the net fitness of the three karyotypes obtained by the methods above with adaptive values calculated from population cage data. The effects

TABLE 7

Relative adaptive values (W) *of karyotypes determined from life cycle components of flies cultured under near-optimal conditions at 15°C*

Life-cycle component	*WT/WT*	*WT/KL*	*KL/WT*	*KL/KL*
Females surviving (30 days) (rate per day)	0.999	1.000	0.996	0.995
Egg-laying capacity (daily average for 30 days)	0.914	1.000	0.957	0.911
Rate of development (egg to adult)	0.924	1.000	0.954	0.773
Adults eclosing per 100 eggs	0.946	1.000	0.991	0.904
Net *W*	0.799	1.000	0.900	0.634
Net *W* (at equilibrium compensated for maternal effect)	0.811	1.00		0.643

of crowding, extreme competition, and the presence of more than one karyotype in a population cage are at least three principal factors which may strongly influence the net fitness values. Some preliminary tests indicate that crowding of larvae tremendously intensifies the heterotic effect in survival.

Table 7 summarizes all factors tested in this project in terms of net relative adaptive values for each life cycle component. The four principal factors, adult female survival, 30-day average egg-laying capacity of females, rate of development, and percent adults eclosing, have been transformed into a rate per day relative to *WT/KL* (the so-called "fly-day" unit of WALLACE 1948). These transformations require further explanation. Rate of female survival was reckoned on the basis of a linear relation between time and percent survival. The data of table 5 follow reasonably a linear relation so that the mortality percent can be divided by 30 and subtracted from 100 to obtain survival per day. Egg-laying is already averaged per day per female. Rate of development was calculated by taking the reciprocal of the total days values in table 6. Adults eclosing can be considered in terms of relative "fly-days" if the time it takes for eclosion of all flies is equal for these karyotypes. This time ranged from six to fifteen days in various cultures and no karyotypes differed significantly in total time. Each proportion of fly-days in table 7 can then be multiplied with the corresponding proportion of the next factor to arrive at a net adaptive value (*W*) for the four main factors tested.

The final values given in table 7 are estimated on the basis of chromosomal frequencies in the population at equilibrium as determined from population cage results. Granted the conditions are not strictly comparable in the cage with these near-optimal "standard" conditions, the only point to be emphasized is this: the less frequent chromosome will be less likely to occur in a mother's genome. When the frequency of *KL* drops to 30% only 15% of all *WT/KL* individuals will have *KL/KL* mothers. In nature where *KL* is even less frequent (7% at White Wolf Pond) *KL/KL* individuals will exist very rarely indeed and nearly all *WT/KL* individuals will have either *WT/WT* or *WT/KL* mothers. Heterokaryotypes with *KL* mothers have consistently lower values than those with *WT* mothers.

By comparing these relative adaptive values with those obtained by population cage methods (above), it can readily be seen that both homokaryotype values are overestimated under these "standard" conditions, although the relative values of *WT/WT* and *WT/KL* are exceedingly close. Either population cage conditions might

intensify these values or factors in the life cycle not yet tested might alter the net results.

A comparison of these facts with those obtained for karyotypes of Standard and Chiricahua chromosomes in *D. pseudoobscura* (LEVINE 1952; MOOS 1955) is worth noting. LEVINE found that when flies of that species from Pinon Flats were raised under near-optimal conditions no differences could be demonstrated in terms of egg viability, egg hatching rate, larval viability, or larval growth rate, although many different food yeasts, temperatures, and relative humidities were employed. Pupae and adults did display slight differences between certain karyotypes. MOOS measured similar life cycle components on the same karyotypes from Pinon Flats but raised them under competitive conditions for direct comparison with population cage calculations of net adaptive values. In contrast with LEVINE's results MOOS found not only substantial differences between karyotype performances but also showed that net adaptive values from population cage competition were reasonably close to values obtained by averaging the life cycle components which she had chosen to measure.

D. persimilis from the Yosemite region raised under near-optimal conditions does display karyotype differences in life cycle components. Whether this fact establishes another physiological contrast with its sibling species or whether it reflects some difference in the ecology and population density of the two in nature cannot as yet be answered. CARSON (personal communication) has stated that the density of these species in breeding sites is an enigma not yet solved.

Heterosis and homeostasis

The heterokaryotype *WT/KL* occurs at a frequency of 0.12 in the locality tested. This represents a low frequency relative to *WT/WT*; nevertheless *KL* has maintained itself in approximately the same proportion in the Yosemite since the region was first collected in 1940. Undoubtedly some or all of these factors could contribute to the balanced condition of these chromosomes in nature: Heterosis is expressed as superiority of heterokaryotype over either homokaryotype under these experimental conditions markedly in some components of the life cycle, namely egg hatchability and rate of third larval instar development, and less markedly in survival of adult females and thirty day average fecundity of females. A lowering of the environmental variance component is demonstrable also especially in egg hatchability and in rate of post-embryonic development. These heterotic effects in the heterokaryotype are above any significant increases of strain cross F_1 homokaryotypes over their parental strains. Maintenance of equilibrium in population cage experiments may well be founded upon life cycle components such as those tested but their intensities may be altered if culture conditions for testing were made to simulate the cage.

WT, the more common karyotype in nature, displays heterosis in rate of development when made heterozygous by strain crossing but *KL* does not. In no other factors tested was there any marked difference between these homokaryotypes. Certainly in such large populations as *D. persimilis* attains in summer at high elevations, heterozygosity at most loci is maintained by the breeding structure. The *WT* arrangement seems to possess heterotic loci not found in the *KL* arrangement which increase rate of development. Loci in the residual genome are just as heterozygous in *KL* F_1's;

apparently then loci determining faster rate of development are on chromosome III to a large extent and act in heterozygotic state either between *WT* arrangements or between *WT* and *KL* arrangements, the latter giving a superior rate.

On the other hand in survival of embryonic and post-embryonic stages both homokaryotypes display equal net survival but are heterotic with respect to their more homozygous parents. Superimposed upon an apparent total genome heterosis, then, is the increased superiority of the heterokaryotype in terms of egg hatchability and adult eclosion. In this case it can be seen that the inversion contains a complex of many genes selected for the totality of expression in heterozygous condition as a supra-genic unit.

Without doubt homokaryotype *WT* must have adaptive superiority over other homokaryotypes occurring in the White Wolf locality. It is too early to speculate about the main causes for the super-abundance of that arrangement and its gradient in relative frequency from low to high elevation, but the results reported above can partially account for the maintenance of a relatively rare arrangement (*KL*) for many generations in the locality.

SUMMARY

1. To test for adaptive differences between karyotype combinations of the Whitney (*WT*) and Klamath (*KL*) arrangements of chromosome III from *D. persimilis* native to an 8000 ft. locality in Yosemite National Park, four strains of each arrangement were tested as well as their strain cross F_1's in homokaryotype and heterokaryotype under near optimal conditions at 15°C for the following life cycle components: 30 day egg-laying capacity, % hatchability, % adults eclosing, rate of development from egg to adult, and survival of adult females.

2. No differences are significant between *WT/WT* and *KL/KL* for egg-laying capacity, hatchability of eggs and net survival of post-embryonic stages.

3. *WT/WT* is superior to *KL/KL* in rate of development in all post-embryonic stages; the former reaches adulthood six days ahead of the latter on the average. *WT* is the most common arrangement in the native locality tested, and this factor may play a major role in maintaining the relative frequencies of these karyotypes in nature.

4. Heterosis is expressed in the heterokaryotype in some components of the life cycle, notably in % hatchability of eggs and in rate of third larval instar development, and less markedly in survival and fecundity of adult females. The advantage gained by high hatchability contributes substantially to high eclosion since mortality of larvae and pupae is about equal for all karyotypes.

5. Karyotypes which express traits which might be termed adaptively superior often show an accompanying lowering of variance in the environmental component, substantiating the hypothesis of developmental homeostasis. This fact is distinctly evident in *WT/KL*'s egg hatchability and total days from hatching to eclosion. When homokaryotypes F_1's are compared with their parental strains heterosis and uniformity occur in *WT/WT* and *KL/KL* for egg hatchability and eclosion, while only in *WT/WT* for rate of post-embryonic stages of development. These results indicate that loci on chromosome III determine faster rate of development when heterozygous

in the *WT/WT* or *WT/KL* karyotype while heterozygous loci throughout the entire genome probably increase viability of eggs and post-embryonic stages.

6. Reciprocal effects in the heterokaryotype are most marked in egg-laying capacity, survival of adult females, and hatchability of eggs; there is no measurable maternal effect on rate of development.

7. A comparison of "net adaptive values" obtained by measuring life cycle components with those obtained by population cage technique shows an overestimation of homokaryotype values with respect to the heterokaryotype, although under these near-optimal conditions the relative values of *WT/WT* and *WT/KL* are exceedingly close. Either population cage conditions or factors in the life cycle not yet tested might alter the net result.

ACKNOWLEDGMENTS

The authors are very grateful to DR. C. C. LI for his generous advice in statistical interpretation. We also wish to thank MARY ROWAN, MARY CLUTTER, JACK CAPENOS, and LOIS HAYWEISER for assistance in the laboratory. The authors wish to express appreciation to MRS. LURETTA SPIESS for her efforts in field collecting and in helpful criticism of the manuscript.

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ON THE INHERITANCE OF NATURAL ANTISHEEP AGGLUTININS IN MICE OF INBRED STRAINS[1]

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Received January 16, 1956

PREVIOUS studies of DAVIDSOHN and STERN (1949, 1950) and of STERN and DAVIDSOHN (1954) have demonstrated significant differences in distribution of titers of natural agglutinins for sheep red cells within and between inbred strains of mice. Results obtained on substantial numbers of mice of 13 strains are summarized in table 1. These mice, which consisted of approximately equal numbers of males and females ranging in age from three to six months, showed greater interstrain than intrastrain differences. This suggested the possible influence of genetic factors. The degree of genetic control of differences in distribution of agglutinin titers is the subject of this investigation.

The two strains selected for the present study, C57BL and C3H, were chosen because of the difference between them in antibody distribution and in several other biologic properties, such as incidence of spontaneous mammary tumors.

MATERIALS AND METHODS

The C57BL and C3H strains investigated are derived from mice obtained in 1946 from DR. A. TANNENBAUM, Cancer Research Department of Michael Reese Research Institute, Chicago, who in turn had bred these strains since 1940 from stock originally obtained from Jackson Memorial Laboratory, Bar Harbor, Maine. The animals of our colony were maintained by brother-sister mating, kept in stainless steel cages with food hoppers, and fed ad libitum Rockland mouse pellets and water. Biologic characteristics, such as fertility, incidence of spontaneous tumors, and longevity of our colonies, compare well with those recorded in the literature for these strains reared elsewhere. Transplantable mammary tumors EO 771 growing in Bar Harbor C57BL were found 100 percent transplantable to our C57BL strain, killing all inoculated animals; the same was true for lymphosarcoma C3HED (Bar Harbor) inoculated into our C3H mice.

Animals of both sexes were tested. Their ages ranged from three to six months. This age limit is important since in previous studies we found that the ability of mice to produce natural antisheep agglutinins is not developed fully until they are from 12 to 15 weeks old (DAVIDSOHN and STERN 1950). This phenomenon of "serologic maturation" applies to natural antibodies in general (HIRSZFELD 1928).

Blood was obtained either by decapitation or by bleeding from the tail. The latter technic permitted repeat testing of the same animals. The technic of testing for

[1] Supported in part by a research grant (C-1113) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, and by a grant from the Howard Faigen Cancer Fund, Chicago, Illinois.

TABLE 1

Natural agglutinins for sheep red cells in mice[1]

Strain	Total no.	Percentage distribution of titers											
		0	1	2	4	8	16	32	64	128	256	512	1024
C57BL	336	9.5	5.7	10.4	12.8	17.0	12.8	13.7	9.7	4.5	2.4	0.9	0.6
C3H	201	73.6	10.4	7.0	4.5	4.0	0.5	—					
C57L	100	71.0	13.0	6.0	8.0	2.0	—						
C57BR/cd	126	59.5	15.9	14.3	5.6	3.9	0.8	—					
STOLI	88	26.1	23.9	10.2	23.9	6.8	8.0	1.1	—				
NH	103	76.7	6.7	8.7	4.9	1.0	0	2.0	—				
BALB/c	103	57.3	16.5	16.5	5.8	3.9	—						
I	91	80.2	12.1	6.6	1.1	—							
A	101	75.2	11.9	7.9	4.0	0	0	1.0	—				
MA	128	55.5	9.4	16.4	14.8	3.1	0.8	—					
DBA	261	43.3	18.8	19.2	9.5	4.2	1.9	2.3	0.8	—			
CBA	120	87.5	7.5	4.2	0.8	—							
AKR	116	66.4	11.2	9.5	8.6	4.3							

[1] Modified from STERN and DAVIDSOHN (1954).

antisheep agglutinins has been reported in previous papers (DAVIDSOHN and STERN 1949, 1950).

Reciprocal crosses of C57BL and C3H mice produced F_1 offspring. Brother-sister mating of F_1 mice resulted in F_2 offspring, and breeding of F_1 females to the male parents, and of F_1 males to the female parents, furnished backcrosses B_1 and B_2.

RESULTS

The distributions of agglutinin titers obtained for parent strains, crosses and backcrosses, are shown in table 2. Data for the parent strains refer to the total population tested instead of to the small number of actual parents of the F_1 mice. For the sake of easier handling of the data, titer was converted into "concentration", indicating amount of the serum in one ml of the highest dilution which yielded agglutination. For convenience, the negative scale, obtained by using the logarithm of the concentration, was converted into a positive scale by multiplying it with minus 10 (table 2). These data, plotted on a log scale (fig. 1), give some evidence that a threshold is involved which would render inappropriate analyses that assume a normal distribution. However, within the range of "positive" titers (one or higher), the data for C57BL showed a normal distribution, suggesting that within this range the two criteria of a satisfactory scale for genetic analysis are satisfied, namely, (1) additivity of genetic factors causing variation, and (2) independence of genetic and non-genetic variation.

Methods for the estimation of the mean and variance of data involving threshold effects have been developed by WRIGHT (1934). These methods involve the construction of an artificial second threshold at some arbitrary point along the scale of observed variation. The two thresholds divide the observed distribution into three percentage classes: (1) values below the lower threshold, representing in this study those animals in which no detectable agglutinin was found; (2) values be-

TABLE 2

Distribution of agglutinins in parents and crosses

Titer		<1	1	2	4	8	16	32	64	128	256	512	1024
Concentration†		>1	1	.5	.25	.125	.0625	.01325	.01562	.00712	.00306	.0019	.00097
$\log_{10}$ concentration			0	−.301	−.602	−.903	−1.20	−1.50	−1.81	−2.11	−2.41	−2.71	−3.01
−10 $\log_{10}$ concentration			0	3.01	6.02	9.03	12.04	15.0	18.1	21.1	24.1	27.1	30.1
		Percentile Distribution											
C57BL	No. 338	33	19	35	43	57	44	46	33	15	8	3	2
	% 100	9.8	5.6	10.3	12.7	16.9	13.0	13.6	9.8	4.4	2.4	0.9	0.6
C3H	No. 205	152	21	14	9	8	1						
	% 100	74.1	9.1	6.8	4.4	3.9	0.05						
F_1	No. 122	53	11	13	19	13	10	2	1				
	% 100	43.4	9.0	10.6	15.6	10.6	8.2	1.6	0.8				
F_2	No. 254	122	22	33	28	22	14	7	4	1	1		
	% 100	48.0	8.7	13.0	11.0	8.7	5.5	2.7	1.6	0.4	0.4		
B_1*	No. 93	27	6	11	7	11	14	11	6				
	% 100	29.0	6.5	11.8	7.5	11.8	15.1	11.8	6.5				
B_2**	No. 141	85	16	17	6	10	5	2					
	% 100	60.3	11.3	12.1	4.3	7.1	3.5	1.4					

† Represents amount of serum in 1 ml of test dilution.

* Backcross of F_1 to C57BL parents.

** Backcross of F_1 to C3H parents.

tween the two thresholds; and (3) values above the higher threshold. The three percentages delimited by these thresholds determine a distribution of unit area without limiting assumptions other than that the data are normally distributed.

Tentatively, let us accept the assumption that on a log scale there is a normal distribution in the parental strains, crosses, and backcrosses, although this is partially obscured by a threshold which prevents observation of part of the variation. Under this assumption and by following the method of WRIGHT, we construct an arbitrary second threshold at a point just greater than nine on the modified log scale. The percentile distributions of parental strains, of crosses, and of backcrosses are shown in table 3, and plotted on a modified log scale in figure 2. If we let the interval zero to nine inclusive on the modified log scale equal one unit on a new arbitrary scale, then each of these percentile distributions defines a unique normal distribution of unit area given by the formulae:

$$pri^{-1}p_1 = \frac{t_1 - m}{\sigma} \quad \text{and} \quad pri^{-1}p_2 = \frac{t_2 - m}{\sigma}$$

where $pri^{-1}p_1$ is the inverse probability integral of p_1, t_1 is the observed threshold, t_2 is the artificial threshold, p_1 is the percentage below t_1, p_2 is the percentage below t_2, and m and σ are the mean and standard deviation, respectively, of the fitted normal distribution as measured in the units of the arbitrary scale.

Since t_1 has a value of zero and t_2 a value of one on the arbitrary scale, the above expressions may be simplified to $\frac{t_1 - m}{\sigma} = \frac{-m}{\sigma}$, $\frac{t_2 - m}{\sigma} = \frac{1 - m}{\sigma}$ and $\frac{1}{\sigma} = pri^{-1}p_2 - pri^{-1}p_1$. Solving these for σ and m, we obtain $\sigma = \frac{1}{pri^{-1}p_2 - pri^{-1}p_1}$ and $m = -\sigma pri^{-1}p_1$.

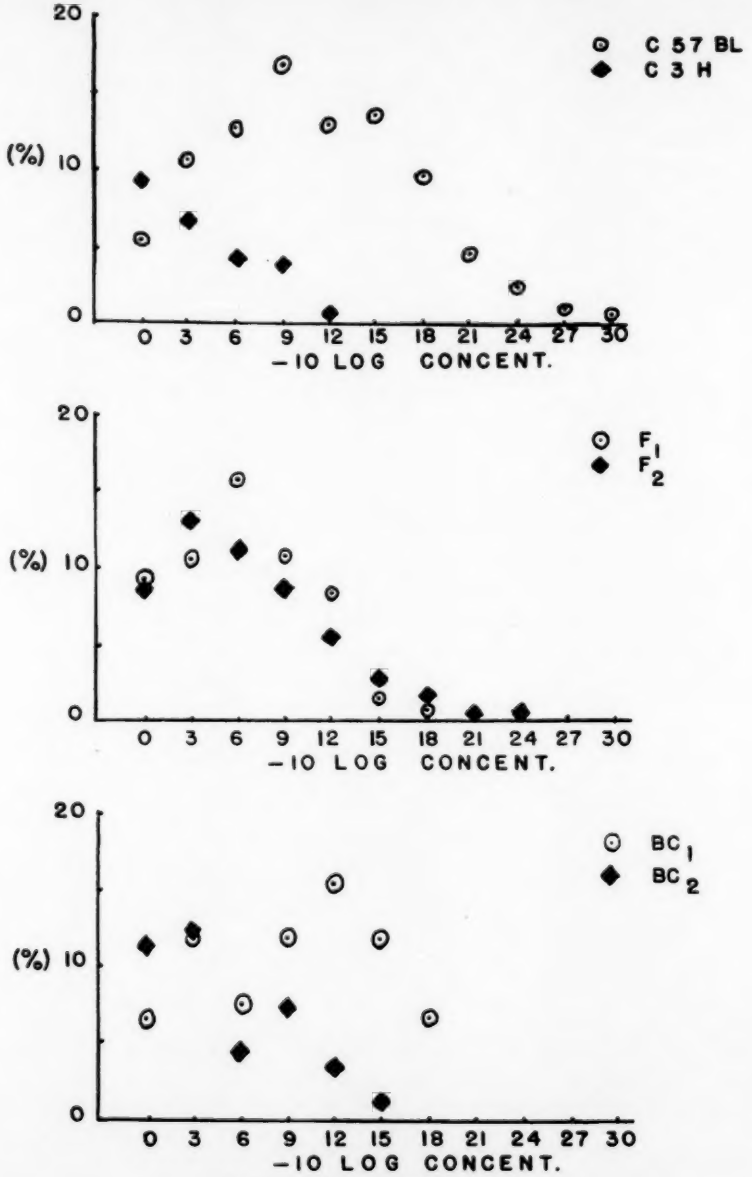

FIGURE 1.—Observed titer distribution.

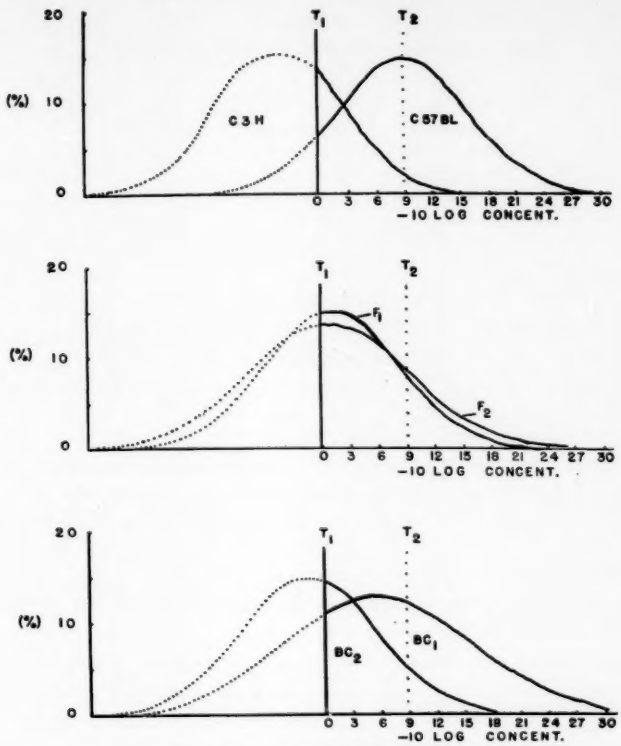

FIGURE 2.—Calculated titer distribution.

TABLE 3

Percentile distribution around two thresholds

	P_1*	P_2-P_1*	P_2*
	$-10 \log_{10}$ concentration T_1		T_2
	0	0-9	9
P_1(C57BL)	9.76	45.56	44.67
P_2(C3H)	74.15	25.37	0.49
$F_1(P_1 \times P_2)$	43.44	45.90	10.65
$F_2(F_1 \times F_1)$	48.03	41.34	10.63
$B_1(F_1 \times P_1)$	29.03	37.63	33.33
$B_2(F_1 \times P_2)$	60.28	34.75	4.96

* P_1 is the percentage of animals without detectable antibodies and may also be considered as the area (fig. 2) under a curve to the left of line T_1 which is the observed threshold. P_2 is the total percent or area to the left of the arbitrary threshold T_2, and $P_2 - P_1$ is the area of percent between thresholds.

TABLE 4

*Means and variances of agglutinins calculated on an arbitrary scale**

Group	Mean	σ	σ^2	σ_G^2
C57BL	0.90657	0.69984	0.48978	
C3H	−0.42500	0.65587	0.43017	
F_1	0.11716	0.70922	0.50299	
F_2	0.04123	0.83808	0.70238	.22807
B_1	0.56205	1.01729	1.03488	.56057
B_2	−0.18772	0.72036	0.51892	.04461

* For explanation see table 3 and text.

The means and variances thus calculated from the data of table 3 are presented in table 4. The calculated distributions, as plotted on the modified log scale, are shown in figure 2. It may be noted that the calculated distributions show fairly good agreement with the observed distributions plotted in figure 1.

Using the values of the means and variances (σ^2) calculated above, we may now test the assumption that the log scale is one on which the two criteria of an adequate scale for genetic analysis are satisfied. We test the additivity of the genetic components of variation, in accordance with the method of MATHER (1949), by defining P_1, P_2, F_1, F_2, B_1 and B_2 as the means of parental strains, crosses, and backcrosses, respectively, and the variances of these variables as $\sigma^2_{P_1}$, $\sigma^2_{P_2}$, $\sigma^2_{F_1}$, $\sigma^2_{F_2}$, $\sigma^2_{B_1}$ and $\sigma^2_{B_2}$, in corresponding order. Accordingly, we express the three functions A, B, and C with their variances:

$$A = 2B_1 - F_1 - P_1 \qquad \sigma^2_A = 4\sigma^2_{B_1} + \sigma^2_{F_1} + \sigma^2_{P_1}$$

$$B = 2B_2 - F_1 - P_1 - P_2 \qquad \sigma^2_B = 4\sigma^2_{B_2} + \sigma^2_{F_1} + \sigma^2_{P_2}$$

$$C = 4F_2 - 2F_1 - P_1 - P_2 \qquad \sigma^2_C = 16\sigma^2_{F_2} + 4\sigma^2_{F_1} + \sigma^2_{P_1} + \sigma^2_{P_2}$$

MATHER has shown that if the genetic components of variation are additive, the values for each A, B, and C will equal zero. The values of A, B, and C, their variances, standard deviations, the ratios of the deviation of the variables from zero to their standard deviations, and the probability of these ratios as unit normal deviates, are shown in table 5. It may be seen that none of these deviations differs significantly from zero. Hence, there is no evidence to suggest non-additive interaction among the loci involved in the control of antibody production.

The second criterion for a satisfactory scale on which genetic analysis can be made is that of independence of the genetic and non-genetic components of variation. It may happen that the environmental variation is reduced or increased within a particular part of the range, or that an increase in genetic variation might cause an increase in sensitivity to non-genetic factors. Under such conditions a correlation between genetic and non-genetic variation might be observed.

The question of the independence of the genetic and non-genetic components may be tested by finding out whether the variances of the two parental strains and the F_1 population are equal. On a satisfactory scale there should not be any genetic variation within each of these three populations since each is genetically homogene-

TABLE 5

Calculated values of functions in additivity test

	A	*B*	*C*
Value	0.10037	2.06760	0.55087
Variance	5.13229	3.00883	14.23768
Standard deviation	2.26546	1.73459	3.77328
Value/standard deviation	0.0443	0.0389	0.1459
Probability	>.5	>.5	>.5

TABLE 6

Calculated values of test for independence of genetic and non-genetic components of variance using null hypothesis of independence ($\sigma_{P_1}^2 = \sigma_{P_2}^2 = \sigma_{F_1}^2$)

Variable	Value	Degrees of freedom	Probability
$F_{P_1F_1}$	1.02699	$F_1$121 $P_1$337	>.20
$F_{P_2F_1}$	1.16930	$F_1$121 $P_2$253	>.20
$F_{P_1P_2}$	1.13858	$P_1$337 $P_2$253	>.20

ous: members of the pure strains are homozygous, and F_1 individuals are uniformly heterozygous at each locus where the parents differ.

Applying FISHER's *F* test (1953), we find, as shown in table 6, that there is no significant difference between the three variances. Hence, we may conclude that the original assumption of independence of the genetic and non-genetic components of variation (cf. table 2 and fig. 2) is justified.

Since each of the parental and the F_1 populations is genetically homogeneous, variation within each of these three populations must be due to non-genetic factors. We may use an average of the three variances as an estimate of the degree of non-genetic variation found within F_1 and backcross populations. The average variance of the parental and F_1 populations, as calculated on the arbitrary scale, is 0.4743 ± .0317. Subtracting this value from each of the individual variances of the crosses and backcrosses, we obtain an estimate of the variance of each of these populations which may be attributed to heredity. These data are shown in the last vertical column in table 4 (σ_G^2).

By comparing the mean and estimated heritable variance of the backcross of the F_1 to C57BL with the mean and variance of the backcross of the F_1 to C3H, we may obtain an estimate of the dominance relations of the genetic factors involved. It must be kept in mind that the differences in the two parental strains are probably due to more than one genetic factor and that the dominance effects observed may be due to interaction of many different genes, each with its own dominance relations. Thus the dominance observed will probably not be complete, but be expressed as an intermediate quantitative effect.

The existence of dominance relations controlling agglutinin titer is clearly suggested by the large differences between the genetic variances of the two backcrosses (table 4). The backcross to C3H has a genetic variance only slightly greater than that of either parental strain, while the genetic variance of the backcross to C57BL is the largest observed in this series of experiments. This suggests that the C3H

parental strain carries genetic factors that are strongly dominant over those of C57BL and act to reduce the titer of antisheep agglutinins.

Confirmatory evidence for the dominant role of the C3H genotype is found in the position of the means of the F_1 and F_2 populations. In the absence of dominance the means of these populations should both fall on the mean of the parental means as calculated on a satisfactory scale. In the presence of dominance the F_1 mean should be closer to the dominant parent, as should be also the F_2, though to a lesser degree. The mean of the parental means as calculated on the arbitrary scale is 0.24076. The observed means of F_1 and F_2 (table 4) are both on the C3H side of this value, confirming the dominance of this parental genotype as found in the analysis of variance. It is an anomaly that the F_2 mean is closer to that of C3H than is that of the F_1, but this may be partially due to restricted sample size.

A method for the estimation of the number of genetic units, which may be blocks of genes acting as one physiological unit, involved in the difference of the two parental strains, has been developed by WRIGHT (1934). His method requires only data for the parental, F_1 and F_2 populations as distinguished from the usually more satisfactory method of MATHER which requires F_3 and double backcross data. WRIGHT's method is critically dependent on several assumptions, the failure of any one of which will cause a great reduction in the estimate of the number of genes involved in the trait studied. These assumptions are: independence, additivity and equality of the genes involved; and that one parental strain contains all the alleles tending to increase the trait while the other contains all the alleles tending to reduce it.

It is evident from table 1 that C3H is not at an extreme of the observable scale of titer variation and hence is not as different in genetic makeup from C57BL, as are some of the other strains tested (e.g., CBA and I). It must, therefore, be recognized that the estimate obtained by WRIGHT's method will be lower than the actual number of genetic units in which the parental strains differ.

Let D be the difference between the parental means measured on a satisfactory scale, and $\sigma^2_{F_2}G$ (cf. table 4) the genetic component of the variance of the F_2 population, and N the number of genetic factors causing difference in the two parental strains. Then, provided that criteria mentioned above are satisfied:

$$N = \frac{D^2}{8\sigma^2_{F_2}G} \quad \text{(WRIGHT)}$$

Solving for N gives a value of 0.9486. Hence, we may conclude that there is a difference between the parental strains which involves at least one genetic factor, possibly many more.

The population data presented are compatible with the hypothesis that the strains differ in a single incompletely dominant factor. Pedigree analysis did not further elucidate this question because of a high "within litter" correlation of backcross mice.

DISCUSSION

There is general agreement in the literature that "constitutional" variation limits the ability of individuals to produce antibodies. WIENER (1951) proposed that

environmental factors, such as subclinical infections, exposure to saprophytes, or food constituents, are responsible for development of "natural" antibodies. Nevertheless, he recognized that these factors act as triggers which stimulate a genetically determined immune response. There is difference of opinion regarding the specificity of gene action: one school proposes direct gene-antibody interaction (FURUHATA 1927), while others suggest various degrees of indirect relationships (SCHIFF and ADELSBERGER 1924; WIENER 1951).

CARLINFANTI (1948) examined titers of isoagglutinins anti-A and anti-B in human parents and their children and found that there was a sufficient parent-child correlation to warrant the assumption that genetic factors control production of these antibodies. His population data showed that the isoagglutinin titer is distributed normally on a logarithmic scale, as would be expected on the basis of a multigene hypothesis with genes acting in a geometric way, as observed in this study.

Several investigations have been concerned with genetic control of immune antibodies. FJORD-SCHEIBEL (1944) observed considerable variation in the ability to produce diphtheria antitoxin in a non-inbred guinea pig colony. She undertook to breed selectively a high-titer and a low-titer strain. The F_1 individuals selected for their high titer contained over 90 percent producing antibody. This percentage remained stable in their offspring for six generations when the experiment terminated. Selection for low titers progressed more slowly. The point at which 90 percent of animals did not produce antibody was not reached until the fifth generation, and was preceded by a gradual decline in frequency of antibody producers. The author considered these results as evidence for a dominant gene controlling production of antitoxin. CARLINFANTI proposed a more likely hypothesis, i.e., that the gene controlling production of antitoxin is recessive, in which case the "high-titer" animals would be homozygous recessives and their offspring would therefore be "high-titer" animals, whereas the "low-titer" individuals with the dominant gene for inhibition of antitoxin production might be heterozygous. This picture of gene action is similar to that reported in the present study of natural antisheep agglutinins in the mouse.

DAVIDSOHN and STERN (1949, 1950, 1954) found striking interstrain differences in titers of immune antibodies for sheep and chicken erythrocytes in mice of 10 inbred strains. With one exception (strain A), mice of strains with high incidence of spontaneous tumors showed lower levels of immune antibodies than did mice of strains with low incidence of spontaneous tumors. Genetic factors may be assumed to play a role in these interstrain variations, as well as in the observation by FINK and QUINN (1953) on strain differences of mice in their ability to produce antibodies for egg albumin and pneumococcus polysaccharides.

In studying genetic control of immune response in rabbits, SANG and SOBEY (1954) reported that the secondary antibody response to tobacco mosaic virus (TMV) depends on inheritable factors. Results of immunization with diphtheria toxoid and beef red cells ran parallel to the immune response to TMV, but this was not the case for other antigens such as bovine albumin and human red cells. Nevertheless, the authors concluded that an inheritable general predisposition for good or poor antibody response existed in rabbits.

Thus, various investigations using different approaches have brought forth evidence for genetic factors controlling at least in part "natural" antibodies in man

(anti-A and anti-B) and in the mouse (agglutinins for sheep red cells), and immune antibodies in guinea pigs, mice and rabbits. A study of immune antibodies for sheep and chicken red cells in hybrids and backcrosses of inbred mice, similar to the present investigation, is underway in our laboratory.

Analysis of the data of this study had to take into consideration the question as to whether presence of the "milk agent" (mammary tumor inciter, MTI) had an effect on distribution of sheep agglutinin. MTI, present in our C3H mice, was presumably transmitted to F_1 offspring with maternal C3H parent, but not in the reciprocal cross, and similarly it was presumably present in backcrosses to the maternal C3H parent. Separate tabulation of data obtained for the reciprocal hybrids and backcrosses failed to reveal any significant differences, and hence the data were pooled for presentation and analysis. Likewise, no difference was observed in antibody dependent on the sex of the hybrids. The failure of the MTI to influence sheep agglutinins is in good accord with results previously reported by DAVIDSOHN, STERN and BITTNER (1950). Furthermore, the similar antibody distribution in reciprocal crosses appears to eliminate the possibility that cytoplasmic inheritance affects formation of antibodies, since this would be expected to lend greater influence to the maternal parent furnishing the large cytoplasm of the ovum.

Finally, mention may be made of strain-dependent differences in mice found in plasma proteins (JOHNSON, ALBERT, PINKUS and WAGSHAL 1954) and in the excretion of amino acids (HARRIS and SEARLE 1953). Total plasma protein, and especially the globulin fractions, were found to drop with advancing age in cancer-susceptible C3H mice, even prior to development of tumor, but not in C57BL mice characterized by low spontaneous tumor incidence. Urinary taurine excretion was ten times as high in C57BL mice as in mice of strains A or CBA. This cumulative evidence points to fundamental differences in protein anabolism and catabolism in mice of different strains, which possibly may also be reflected in production of antibodies.

It is tempting to suspect a relationship among these biologic properties, and the resistance or susceptibility of mice to cancer, since genetic factors are operative in both types of phenomena. Analyses of genetic factors in incidence of mouse tumors by HESTON (1944) brought out data similar to the classic observations of WRIGHT (1934) on polydactyly in guinea pigs, interpreted as involving polygenic inheritance with a physiologic threshold, similar to the mechanism suggested in the present study for inheritance of natural agglutinins for sheep red cells.

At present the evidence is certainly insufficient for permitting any generalization about the genetic relationship between antibody production and tumor formation. If such a relationship exists, it probably operates on the level of interaction of gene products rather than of the genes themselves, a possibility that may deserve further study.

SUMMARY

(1) Breeding experiments have demonstrated a genetic difference in the ability of mice of strains C3H and C57BL to produce natural agglutinins for sheep erythrocytes.

(2) The differences in agglutinins have been analyzed according to methods of WRIGHT and MATHER, and it has been shown that they are controlled by one or more genetic factors.

(3) The genetic factors associated with low or absent antibody levels are incompletely dominant over those which allow the production of antibodies.

(4) In addition to genetic factors, a strong independent environmental effect was found to affect the antibody distribution.

(5) The findings of this study are discussed in relationship to observations by other authors on genetic control of antibody production and in connection with other biologic differences found in inbred mice, with particular reference to incidence of spontaneous tumors.

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A MAP OF LINKAGE GROUP VI OF NEUROSPORA CRASSA

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Received January 19, 1956

THE linkage maps of *Neurospora crassa* compiled and prepared by BARRATT *et al.* (1954) provide a useful summary of our knowledge of this subject up to the present time. They also point up two serious shortcomings in the linkage data that have been gathered on this organism. At the time these maps were prepared, some 80 mutant loci had been demonstrated to lie in one or the other of the seven linkage groups. There was, however, very little reliable evidence as to which arm of a linkage group contained a particular locus. Indeed, in only one of the seven groups was there conclusive evidence that markers were known in both arms. This lack of knowledge is surprising in Neurospora, an organism in which information about the position of the centromere can be obtained in straightforward genetic tests. A second major flaw in our mapping data is that in most cases in which we know genes to be closely linked, we do not have reliable information about their order of arrangement on the chromosome.

When a new mutant is found to be in a particular linkage group, one way of determining which chromosome arm contains this locus is to examine a cross of this mutant to a known linked mutant. Asci in which the mutant of known location has recombined with its centromere are studied to determine whether there has been coincident recombination between the two mutant loci. This method will be discussed in greater detail in a later section. The point to be made here is that such tests have seldom been carried out in the past because they require asci with 2nd division segregation for the mutant of known location. The known mutants which have been employed in linkage studies have nearly always been very close to the centromere, and thus the test for chromosome arm location has required a rare meiotic event.

The placing of mutant loci on the maps of BARRATT *et al.* is based on centromere distances (2nd division segregation frequencies). In many cases the number of asci studied has been small and the standard error large, so that the range of possible location overlaps with that of a neighboring marker, and the order is not known with certainty. The conviction is implicit, however, that with enough data on 2nd division segregation frequency the markers could be accurately placed along the linkage map. This conviction rests on the assumption that a given mutant will have the same frequency of 2nd division segregation in any cross. Experimental data do not substantiate this assumption. BARRATT (1954) himself has pointed out a number of cases in each of which a given mutant has shown distinctly different frequencies of 2nd division segregation in two different crosses, and he has suggested that these variations in crossover frequency result from some difference between two original wild stocks from which many of our current Neurospora stocks were derived. Studies

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TABLE 1

Frequencies of 2nd division segregation for asco

Cross	1st division segregations	Alternating 2nd division segregations	Symmetrical 2nd division segregations	% 2nd division segregation
A (I)	5020	280	276	10.0%
B (1)	4157	300	297	12.6%
C (9)	89	11	7	16.8%
D (8)	338	40	39	18.9%
E (5)	793	86	106	19.5%
F (6)	553	53	88	20.3%
G (II)	4026	523	536	20.8%
H (3)	481	62	90	24.0%
J (4)	206	28	37	24.0%
K (2)	1066	199	194	26.9%
L (7)	218	49	50	31.2%
M (III)	513	133	131	34.0%
N	283	85	80	36.8%
O	183	60	74	42.3%
P	120	58	53	48.1%
Q (IV)	329	235	229	58.4%

Counts of asci showing the different patterns in crosses segregating for the mutant *asco*. An Arabic number in parentheses after the letter designating a cross signifies that the data are from the cross bearing that number in table 3. Roman numerals indicate the crosses represented by the same symbols in figure 1.

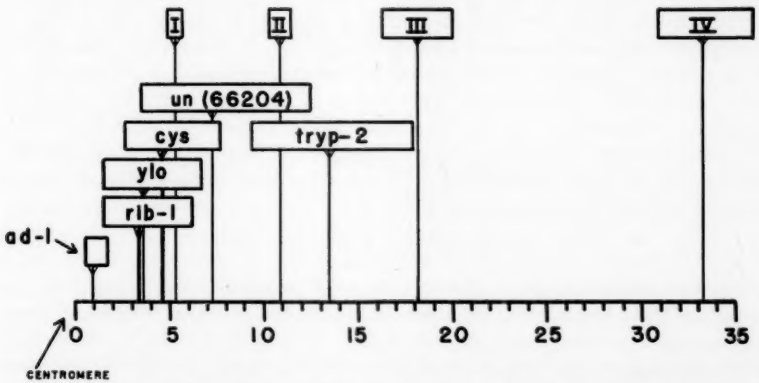

FIGURE 1.—A map of linkage group VI mutants based on 2nd division segregation frequencies. The vertical lines show the map distances from the centromere as calculated from the 2nd division segregation frequencies, and the horizontal bars indicate the limits of statistical fluctuation (plus or minus twice the standard error) of these figures. The loci shown in the lower part of the map (those with the mutant symbols in the horizontal bars) are copied directly from the map of group VI prepared by BARRATT *et al.* (1954). The four points designated by roman numerals are measures of 2nd division segregation frequency for the ascospore mutant, *asco*, in four different crosses. All map distances are corrected for interference by the mapping function curve on page 16 of BARRATT *et al.* (1954).

now in progress with an ascospore mutant (STADLER 1956) and with other mutants lead to the conclusion that there are a large number of separately heritable factors affecting crossover frequency which occur generally in stocks now in use. Until more is known of the nature and distribution of these factors, the assignment of absolute crossover values for regions of chromosome maps would seem to be of limited significance. Counts on a large number of crosses involving the ascospore mutant have given 2nd division segregation frequencies for this locus as low as 10% and as high as 58% and all levels between (table 1). In any given cross, the frequency is constant. Figure 1 demonstrates how this mutant might be placed in any of several quite different locations depending on what cross was analysed. It becomes clear then, that, as in other organisms, the order of neighboring loci can only be determined with confidence if they are studied simultaneously in the same cross. This requires three point crosses (involving three linked mutants or two mutants and the centromere).

The preparation of the linkage map reported in this study has involved two steps: 1) location of all mutants with respect to a given marker and to the centromere; 2) arrangement of neighboring loci by means of three and four point crosses.

LOCATION OF MUTANT LOCI WITH RESPECT TO A GIVEN MARKER AND TO THE CENTROMERE

The method used in the present study has been to cross the linked mutant of unknown location to the given marker and to select those asci in which there has been a crossover between the given marker and the centromere. The extent of recombination between the two mutants has then been determined for this group of asci. (In some crosses, asci with no crossover between the given marker and the centromere have also been analysed.) The method requires that the given marker be close enough to the centromere to insure that there will seldom be more than a single crossover in this region, yet far enough to allow recovery of a reasonable number of crossover asci. The requirement is eminently satisfied by a gene controlling an ascospore character which is closely linked to the centromere, because asci with crossing over between this locus and the centromere can be recognized by their spore patterns, without the labor of dissection.

Ascospore mutant 37402

It was pointed out to the author by MRS. M. B. MITCHELL that GOOD (1951), in a study of lysine-requiring mutants of Neurospora, had observed that one of them, 37402, caused delayed ascospore maturation. On the basis of this information, lysine-requiring mutant 37402 was crossed to wild type and the cross was examined nine days after fertilization. Each ascus which contained any mature, black spores was seen to have two pairs of these and two pairs of colorless ones (figure 2). When such a cross was aged, an occasional ascus was found with a fifth spore maturing, which, when grown, always required lysine and carried the ascospore mutant character. Attempts to separate these two phenotypic expressions by crossing over have failed.

37402 (hereafter referred to as *asco*) is really an ascospore lethal mutant. Most of the spores carrying it never mature. Attempts to bring them to maturity in high

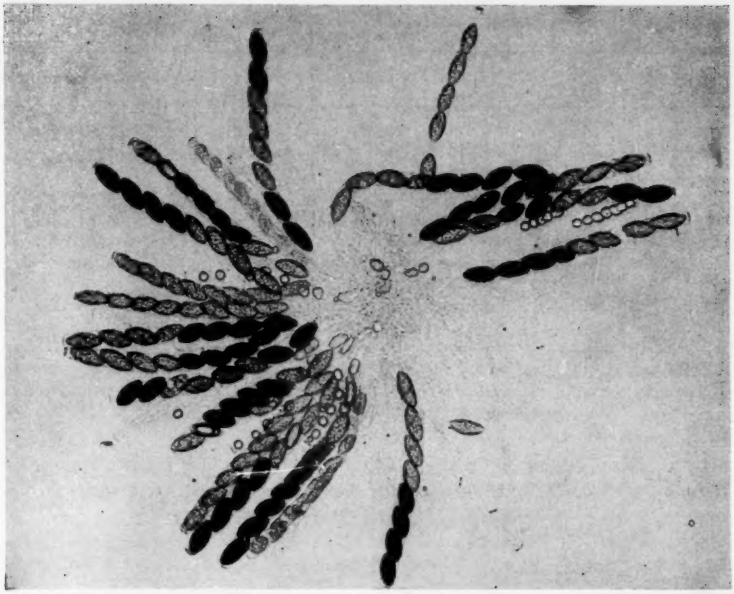

FIGURE 2.—Photomicrograph of asci from a cross segregating for the ascospore mutant, 37402. The mature asci include nine showing 1st division segregation for the ascospore character, one with the alternating pattern of 2nd division segregation and four with symmetrical 2nd division segregation patterns.

lysine medium or other supplemented media have been unsuccessful. This means that in each ascus of a cross segregating for *asco* only the two spore pairs carrying the wild allele can be grown and tested for other markers.

Segregation patterns of *asco* were first studied in a cross to wild strain Abbott 4A. Counts of 2nd division segregation frequency for *asco* were made on 5576 asci of this cross and consistently gave a value of about 10% (cross I of table 1), indicating the locus of *asco* was about five crossover units from the centromere (in this cross). Crosses were then made between *asco* and biochemical mutants close to the centromeres of the various linkage groups. Random spores of these crosses were germinated on minimal medium. Nearly all germinating spores in such a cross carry the wild allele of *asco*, and, if the other mutant involved is not linked to *asco*, half of the germinating spores should exhibit the restricted growth characteristic of a biochemical mutant on unsupplemented medium. If the second mutant is linked to *asco*, more than 50% of the germinating spores will be mutant. In the cross 37402a × 51602A (riboflavin-requiring mutant at 35°C, wild at 25°C), 92.8% of the germinating spores carried the riboflavin requirement, indicating that the ascospore mutant was located in linkage group VI (group B of HOULAHAN *et al.* 1949).

Eight mutants known to be in linkage group VI were crossed individually to *asco*.

TABLE 2

Mutants of linkage group VI

Mutant symbol	Isolation number	Phenotype	Published centromere distance
tryp-2	75001	requires tryptophane	13.5 map units
rib-1	51602	requires riboflavin at 35°C	3.4 m.u.
ad-1	3254	requires adenine	0.9 m.u.
ylo	Y30539y	yellow conidia	3.6 m.u.
cys-1	84605	requires cysteine (or methionine)	4.6 m.u.
un	66204	no growth at 35°C	7.3 m.u.
cyt	C117	slow growth; altered cytochrome system	16.4 m.u. from *rib-1*
cys-2	38401 80702	requires cysteine (or methionine)	0.8 m.u. from *cys-1*

The information on the first six mutants listed is from BARRATT *et al.* (1954) and references there cited. The information on *cyt* is from MITCHELL *et al.* (1953). The linkage information on *cys-2* is calculated from PITTENGER (1954).

(Biochemical and genetic information about these mutants is summarized in table 2.) The crosses were analysed to place the other mutant loci with respect to *asco* and to the centromere. The *asco* locus was arbitrarily placed in the left arm.

Expected types of asci

Figure 3 illustrates the most probable meiotic events which would result in the various ascus types in a cross of *asco* to a linked mutant. The assumption is made that the *asco* locus is close enough to the centromere to insure that there will not be more than a single crossover in this region. This means that all asci with a 1st division segregation pattern for the spore character have had no crossing over between that locus and the centromere, and those with a 2nd division segregation pattern have had one and only one crossover in that region. In some crosses it is possible to detect double crossovers between *asco* and the centromere, and these have been observed to occur with a low frequency. However, they are so rare that they do not seriously affect the analysis.

Figure 3a shows the expected ascus types in a cross between *asco* and a linked mutant in the opposite arm at a position such that there is usually either no crossover or a single crossover between this locus and the centromere. In this cross we can detect crossing over in "region I" (*asco* to centromere) and "region II" (centromere to linked mutant). Examination of the viable (*asco*$^+$) spore pairs from asci showing 1st division segregation patterns for *asco* (no crossover in region I) will reveal no recombination (both pairs mutant) in those cases in which there has been no crossover in region II. Single recombination (one pair mutant, one pair wild) will be observed if there has been a crossover in region II. Asci with the 2nd division segregation patterns for *asco* (single crossover in region I) will show single recombination between the mutant loci if there has been no crossover in region II or if there has been a crossover in this region making a 3-strand relationship with the one in region I. A 2-strand double crossover between the two regions will result

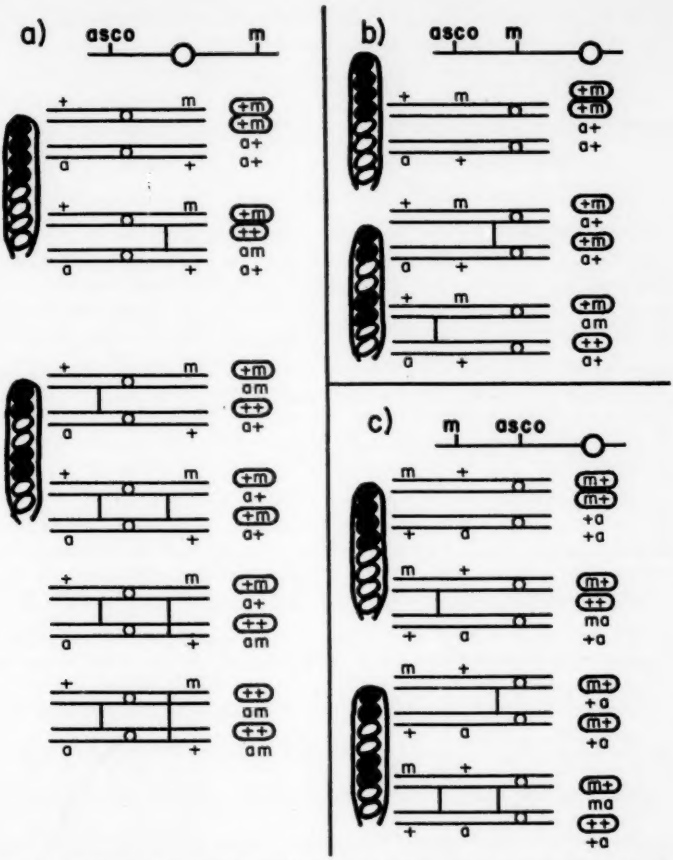

FIGURE 3.—Patterns of segregation resulting from simple crossing over events in a cross between *asco* and a linked mutant. Only one pattern is shown for each situation; patterns which might be achieved by reversing the two spore pairs in either half of an ascus or by reversing the whole ascus are grouped together in the analysis. Ovals are drawn around the genotypes of spore pairs which, since they carry the wild allele of *asco*, can be checked for the presence of the other segregating mutant.

in a non-recombination ascus, and a 4-strand double crossover yields a double recombination ascus (both pairs wild). It is clear that among the asci showing 2nd division segregation for *asco* in such a cross, we would expect the majority to have single recombination between the mutant loci, with the less frequent non-recombination and double recombination types occurring about equally often (assuming no chromatid interference across the centromere). Such a situation is illustrated in the results (table 3) in the cross between *asco* and *tryp-2*.

TABLE 3

Crosses of asco *to linked mutants*

Mutant crossed to *asco*		Map distances		Classification for linked mutant of viable (*asco*°) spore pairs of:						Position of mutant locus
		asco to centromere	*asco* to linked mutant	asci showing 1st division segregation for *asco*			asci showing 2nd division segregation for *asco*			
				m m	*m* +	+ +	*m m*	*m* +	+ +	
rib-1	(1)	6.3 m.u.	7.2 m.u.	101	2	1*	4†	395	1	opposite arm from *asco*
	(T)	8.5–26.0					7‡	474	8	
tryp-2	(2)	13.5	27.5	68	32	1§	12	105	9	opposite arm from *asco*
ad-1	(3)	12.0	11.0				3	78	0	near centromere, arm un
	(T)	12.0–17.5					1	92	1	determined
ylo	(4)	12.0		26	0	0	11	22	0	in same arm, proximal to *asco*
cys-1	(5)	9.7	3.1				27	18	0	in same arm, proximal to *asco*
cys-2	(6)	10.2	7.3				28	32	0	in same arm, proximal to
	(7)	15.6					22	11	0	*asco*
un	(8)	9.5	1.7	36	0	0	24	1	0	near *asco*, probably proxi-
	(T)	8.5–17.0					48	5	0	mal to it
cyl	(9)	8.4	6.5	16	1	0	16	0	0	in same arm, distal to *asco*

Map distances listed for the interval between *asco* and the centromere are the frequencies of 2nd division segregation for *asco* (table 1) divided by two. Map distances between *asco* and the linked mutants were determined by counts of wild growers among germinating random spores on minimal medium.

Lines labeled (T) contain data totalled from several crosses involving *asco* and the mutant under study. Values given in these lines for the map distance from *asco* to the centromere represent the range among the crosses involved.

* This ascus probably resulted from a four-strand double crossover between *asco* and the centromere.

† Three of these asci probably resulted from meiotic nuclear passing with no crossing over in the marked regions.

‡ One of these asci may have resulted from meiotic nuclear passing with no crossing over in the marked regions.

§ This ascus resulted from a four-strand double crossover either between *asco* and the centromere or between the centromere and *tryp-2*.

The expected ascus types in a cross between *asco* and a linked mutant located between *asco* and the centromere are shown in figure 3b. The segment from *asco* to the linked mutant now becomes region I, and from the linked mutant to the centromere is region II. Asci showing 1st division segregation for the ascospore character have no crossing over in either marked region and thus no recombination. Asci with 2nd division segregation for *asco* have a single crossover which can be in either marked region. If it is in region I, they will show single recombination; if the crossover is in region II, there will be no recombination. The percentage of single recombination asci in this group can be anything from 0 to 100 depending upon the position of the linked mutant along the interval from *asco* to the centromere. This type of cross is well illustrated in the results (table 3) in the cross between *asco* and *ylo*.

In the cross to a linked mutant located in the same chromosome arm as *asco* and distal to it, the expected ascus types would be those shown in figure 3c. Single recombination and non-recombination asci may both occur, whether there is 1st or 2nd division segregation for *asco*. However, if there is crossing over interference between the two regions, the frequency of single recombination asci may be expected to be lower among those showing 2nd division segregation for *asco*.

RESULTS

The results of the crosses of the eight linked mutants to *asco* are given in table 3. The analysis of the crosses to one mutant, ***rib-1***, will be described in some detail. The ***rib-1*** locus is clearly very close to the centromere, but an extensive analysis is required if this cross is to tell which arm contains the locus. In the first cross listed, 400 asci with the 2nd division segregation pattern for *asco* were tested, and 395 had a single recombination. Of the remaining five, four had no recombination, but in three of these it could be shown, by means of other segregating markers, that the 2nd division segregation pattern probably resulted from nuclear passing at meiosis rather than crossing over. The method used for this test is very similar to that described by HOWE (1954), and the experiment will be described in greater detail elsewhere. It is sufficient here to state that the evidence is convincing that among 397 asci with crossing over between *asco* and the centromere, all except two had single recombination between the mutant loci. These two include one double recombination and one non-recombination ascus. In the other crosses studied between *asco* and ***rib-1***, crossing over was more frequent in the region between the centromere and the ***rib-1*** locus. There were seven non-recombination and eight double recombination asci among 489 showing 2nd division segregation patterns. One of the seven non-recombination asci may have resulted from nuclear passing with no crossing over (though here the test was less efficient). This gives a total of seven or eight non-recombination asci and nine double recombination asci among those in which *asco* segregated in the 2nd division in all crosses to ***rib-1***. If ***rib-1*** were in the opposite arm from *asco*, the non-recombination asci would be those in which a 2-strand double crossover took place across the centromere, while the double recombination asci would result from the 4-strand double crossovers. If, on the other hand, ***rib-1*** were in the same arm as *asco*, then the double recombination asci could only result from a triple crossover (a 4-strand double between *asco* and ***rib-1***, and a single between ***rib-1*** and the centromere); non-recombination asci would result every time there was a crossover between ***rib-1*** and the centromere with no crossover (or a 2-strand double) between *asco* and ***rib-1***. It is highly improbable that these two events would be equally frequent. The evidence is thus compelling that ***rib-1*** is in the opposite arm from *asco*.

In the cross *asco* × ***tryp-2*** no test was made for nuclear passing in meiosis. The proportion of double crossover asci was so high that the results could only be obscured by aberrant meiotic events occurring much more frequently than has been observed in other crosses.

There was no evidence of meiotic nuclear passing among the four non-recombination asci in the cross to ***ad-1***. The data are not sufficient to determine which arm

contains the *ad-1* locus. If it is in the same arm as *asco*, the double recombination ascus must represent a triple crossover. If *ad-1* is in the opposite arm, then the one double recombination ascus and the four non-recombination asci result from equally probable events, if there is no chromatid interference across the centromere (HOWE 1954; STADLER 1955).

In the cross of *asco* to *cyt*, the analysis of asci tells us that *cyt* is in the left arm but gives no convincing evidence as to whether it is proximal or distal to *asco*. The correct location, as between these two alternatives, is indicated by the distance between the mutant loci as measured by random spore analysis. If *cyt* were 6.5 map units proximal to *asco* (which itself is only 8.4 units from the centromere in this cross), the majority of the 2nd division segregation asci would have a recombination between the mutants. The random spore distance between the mutant loci was also employed in determining the position of *cys-1* and of *cys-2*. In both of these cases the analysis of asci demonstrated that the mutant locus was either between *asco* and the centromere or a long distance beyond it in the same arm.

The cross *asco* × *cyt* required a special method. Both *asco* and *cyt* are relatively infertile. *Asco* can be made to act as a protoperithecial parent only with difficulty, and *cyt* has never been made to serve in this capacity. All attempts to get a pure cross of *asco* and *cyt* failed. The cross was finally made by inoculating both *asco* and a strain of *ad-1* of like mating type in a tube of unsupplemented mating medium. This gave a heterocaryotic growth which produced protoperithecia and was successfully fertilized with *cyt*. When the cross matured, only those perithecia containing asci segregating for *asco* were selected for study. The technique of mixing in a third strain to achieve a cross between parents of poor fertility has been used before (BEADLE and COONRADT 1944; LEIN and LEIN 1952). The advantage of the present method is that by using an ascospore marker in one of the two parent strains of like mating type, it is possible to select the progeny of the desired cross without laborious dissection and growth tests.

At this point it may be worthwhile to make a tentative map (fig. 4) of linkage group VI based on the results of the crosses of the other markers to *asco*. No measure of absolute units is suggested, and even the order of arrangement of the mutant loci is not yet established, but will be determined by the three-point crosses in the following section. The positions on this map of the markers between *asco* and the centromere are derived from the frequencies of recombination in asci showing 2nd division segregation for *asco*. *Rib-1* and *tryp-2* are placed according to their 2nd division segregation frequencies in asci showing 1st division segregation for *asco*. *Ad-1* is placed at the centromere pending further evidence.

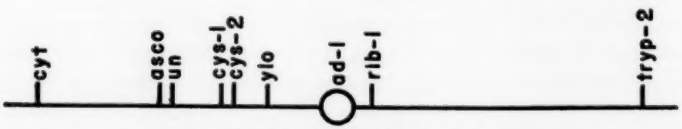

FIGURE 4.—Described in text.

DETERMINATION OF ORDER OF NEIGHBORING LOCI

Crosses involving two mutant loci and the centromere

If two mutant loci are known to be in the same chromosome arm, their order of arrangement with respect to the centromere can frequently be determined by a study of asci, in which there has been recombination between the mutant loci. There are two marked regions in such a cross: region I, bounded by the two mutant loci, and region II, extending from the proximal mutant locus to the centromere. Recombination asci are those with crossing over in region I. If region II is short enough so that there is usually no coincident crossover in this region, the majority of the recombination asci will show 1st division segregation for the proximal mutant and 2nd division segregation for the distal one. A number of the mutants in linkage group VI have been placed by this type of analysis, summarized in table 4.

There has been no direct test of the relative positions of *un* and *cys-1*. A cross between them gave few viable complete asci, and no double mutant progeny were recovered. The cross was discarded. It has been tentatively assumed that *cys-1* is

TABLE 4

Analyses of crosses involving two linked mutants and the centromere

Cross	Non-recombination asci		Single recombination asci		Order of loci
	1st division segregation for both mutants	2nd division segregation for both mutants	2nd division segregation for one mutant	2nd division segregation for both mutants	
1. *un* × *cys-2*	35	8	4 MI seg. for *cys-2*; MII seg. for *un*	0	*un*—*cys-2*—cent.
2. *cys-2* × *ylo*	67	2	3 MI for *ylo*; MII for *cys-2*	0	*cys-2*—*ylo*—cent.
3. *cys-1* × *ylo*	37	3	3 MI for *ylo*; MII for *cys-1*	0	*cys-1*—*ylo*—cent.
4. *ylo* × *ad-1*	106	0	14 MI for *ad-1*; MII for *ylo*	0	*ylo*—*ad-1*, cent.
5. *rib-1* × *tryp-2*	129	4	62 MI for *rib-1*; MII for *tryp-2*	*1*	cent.—*rib-1*—*tryp-2*
			2 MI for *tryp-2*; MII for *rib-1*		
6. *rib-1* × *tryp-2*	8	2	14 MI for *rib-1*; MII for *tryp-2*	0	
7. *rib-1* × *tryp-2*	18	1	21 MI for *rib-1*; MII for *tryp-2*	0	

No double recombination asci were observed in any of these crosses.

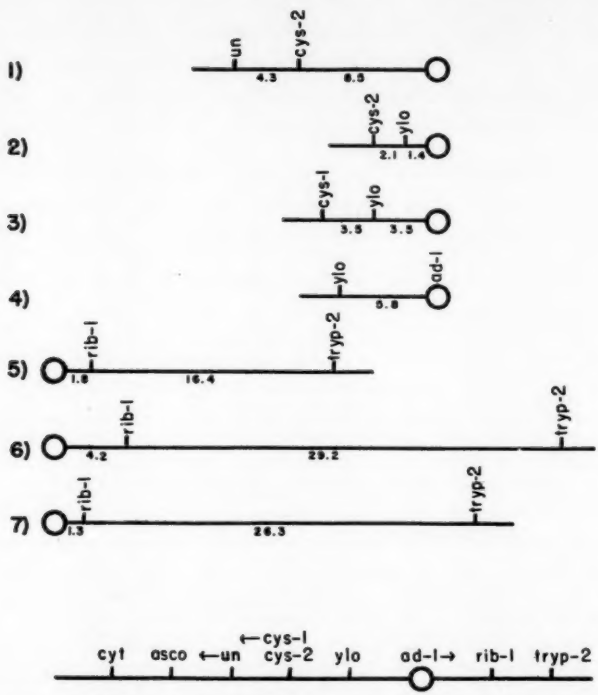

FIGURE 5.—Above: Maps of the results of the seven crosses listed in table 4. Below: A map illustrating what has been determined about the relative positions of the nine mutants and the centromere up to this point. An arrow emanating from a mutant symbol means the position of this locus with respect to the next neighboring mutant has not been established with certainty.

to the right of *un*, as *cys-1* has consistently shown closer linkage to *cys-2* than has *un*. A cross to be described in a later section reveals *cys-1* to be to the right of *cys-2*, thus obviating the study of the relative positions of *un* and *cys-1*.

The cross *ylo* × *ad-1* does not reveal which chromosome arm contains the *ad-1* locus, as this mutant failed to separate from its centromere in any of the 120 asci. The cross demonstrates only that *ad-1* is to the right of *ylo*.

The three *rib-1* × *tryp-2* crosses were also segregating for *asco*, and thus only the two spore pairs carrying the plus allele of *asco* in each ascus could be classified for *rib-1* and *tryp-2*. The observations reported here were confined to those asci showing 1st division segregation for *asco* (as these gave more information about the centromere relations of the other mutants). The data therefore represent a selected group of asci. However, if there is no interference across the centromere (HOWE 1954; STADLER 1955), this type of selection should not alter the apparent linkage relationship of *rib-1*, *tryp-2*, and the centromere.

The data listed in table 4 are shown as partial chromosome maps in figure 5.

TABLE 5

Analyses of crosses involving three linked mutants

Cross	Germination conditions	Phenotype selected	Phenotype for unselected marked	Total germinated spores
un × *asco cys-2*	methionine at 35°C	*un*$^+$ *asco*$^+$	17 *cys-2*, 3+, 6 pseudowilds (+)	1611
ad-1 × *cys-2 rib-1*	methionine at 35°C	*ad-1*$^+$ *rib-1*$^+$	18 *cys-2*, 5+	1920

These results demonstrate again (as was shown earlier for the frequencies of 2nd division segregation for *asco*) that a given chromosome region may show markedly different rates of crossing over in two different crosses. At the bottom of figure 5 is a map summarizing what has thus far been determined about the relative positions of the mutant loci.

Crosses involving three mutant loci

If the relative positions of two closely linked mutants, *a* and *b*, are unknown, but they are both known to be on the same side of another nearby mutant, *c*, then it is possible to determine the relative positions of the loci by a study of the cross *a c* × *b*. Random spores are germinated on selective medium that allows growth of only those which are a^+, b^+ (but does not select between *c* and c^+). Growing colonies are then picked up and classified for *c*. If the *b* locus lies between *a* and *c*, most of the growers will carry the mutant *c*, while if *a* is between the other two mutant loci, most of the growing colonies will carry the wild allele of *c*.

This method was used in the crosses recorded in table 5. The first cross demonstrates that *un* is to the right of *asco*, and the second shows that the *rib-1* locus is to the right of *ad-1*.

Those colonies which express the wild phenotype for all three segregating mutants in these crosses could be pseudowilds (MITCHELL *et al.* 1952) rather than products of crossing over in the marked region. Such cultures were regularly tested for presence of the parental mutant genes by crossing to wild type or by growing and testing macroconidia which failed to grow on minimal medium (PITTENGER 1954). The inclusion of undetected pseudowilds in the data could obscure the results of an experiment of this type.

The position of the ad-1 locus

Experiments described above have demonstrated that the *ylo* locus is in the left arm, *rib-1* is in the right arm, and *ad-1* lies between them. But there has been no decisive evidence as to the position of *ad-1* with respect to the centromere. In order to settle this point, *rib-1* was crossed to *ylo ad-1*, and ordered asci were analysed in the hope of finding one segregating in the 2nd division for *ad-1*. The expectation was that in such an ascus *ad-1* would almost certainly recombine with the mutant which was in the opposite arm while not recombining with the mutant in the same arm.

Among 146 asci studied, 143 had no recombination in the marked region—parental ditypes with 1st division segregation for all three mutants. Two asci segregated in the 2nd division for *ylo*, and there was one ascus with 2nd division segregation for *rib-1*. *Ad-1* segregated in the 1st division in every ascus.

Studies of 2nd division segregation frequencies for *asco* in crosses incubated at different temperatures have revealed that crossing over in the region between *asco* and the centromere is two to three times more frequent among mature asci reared at 18°C than among those grown at the customary 25°C. There is some indication that the effect is most pronounced in the region of the chromosome near the centromere. The effect appears to be just as marked if the cross is at 18° only four days, during a "sensitive period" (fertilization on the 5th day, 18° from the 9th to the 13th day). It is not yet known whether the observations result from a direct effect of the altered temperature on the meiotic apparatus. In any case, it provides a useful method of increasing the yield of recombinant progeny.

The same strains of *rib-1* and *ylo ad-1* were again crossed and were put for a part or all of the incubation period at 18°. Analysis of 64 asci revealed two in which *ad-1* segregated in the 2nd division. Both asci were of the following constitution:

1st spore pair:	*ylo*	*ad-1*	*rib-1*
2nd spore pair:	+	+	*rib-1*
3rd spore pair:	*ylo*	*ad-1*	+
4th spore pair:	+	+	+

This reveals that the *ad-1* locus is in the left arm.

The relationship between cys-1 and cys-2

Crosses involving *cys-1* and *cys-2* and the neighboring markers *ylo* and *un* are reported in table 6. Classification of the cysteine-independent progeny for the unselected markers reveals that, in every case, the most numerous class is that which would result from a single crossover between the *cys* loci if the order of arrangement on the chromosome were *un—cys-2—cys-1—ylo*. Further evidence that *cys-2* and *cys-1* are at separate loci arranged in this order is gained from a study of whole asci of the cross *un cys-2* × *cys-1 ylo*. Dissected asci were germinated on minimal agar plates at 25° and examined after 15–20 hours. Of 43 asci, 42 had all spore pairs showing the restricted growth characteristic of cysteine requirement. The other ascus contained one wild growing pair. The four spore pairs of this ascus were picked up on complete agar slants and classified for all segregating mutants with the following result:

1st spore pair:	+	+	*cys-1*	*ylo*	a
2nd spore pair:	*un*	*cys-2*	*cys-1*	*ylo*	—
3rd spore pair:	+	+	+	+	A
4th spore pair:	*un*	*cys-2*	+	+	A

The genotypes are those which would be expected if the loci were arranged as shown, and a single crossover took place between *cys-2* and *cys-1*, involving the 2nd and 3rd spore pairs. The presumed double mutant (*cys-2 cys-1*) was identified

TABLE 6

Crosses between the two cys *mutants*

Cross	Germination conditions	Phenotype selected	Phenotype for unselected markers	Total germinated spores
cys-2 × *cys-1 ylo*	minimal medium	*cys*$^+$	14 +, 8 *ylo*	2420 (approx.)
cys-2 × *cys-1 ylo*	minimal medium	*cys*$^+$	8 +, 2 *ylo*	1425 (approx.)
cys-1 × *cys-2 ylo*	minimal medium	*cys*$^+$	4 +*, 11 *ylo*	6054
un cys-2 × *cys-1 ylo*	minimal at 25°C	*cys*$^+$	16 +, 2 *un*, 1 *ylo*	1330 (approx.)

* These four cultures tested negative for pseudowild.

only by its vegetative growth habit. In liquid methionine medium at 25° it grew distinctly less than either parent mutant. Perhaps the best test for identification of a double mutant between two closely linked mutants of similar phenotypes is made by studying the backcrosses to both parent mutants (MITCHELL 1955). Unfortunately this test could not be made, as the presumed double mutant was completely sterile.

The above evidence indicates that the *cys-2* and *cys-1* loci are separable by crossing over. However, some of the cysteine-independent progeny in the crosses between the two *cys* mutants could not result from a single crossover. In the first cross listed in table 6, random spore analysis reveals *ylo* to be 7.9 map units from the *cys* loci. To account for the phenotypes of the cysteine-independent progeny by crossing over, we must assume that among the isolates with exchange between the *cys* loci, 8 of 22 also crossed over in this short neighboring region. This unexpectedly high frequency of *ylo* among the cysteine-independent progeny is reminescent of the behavior of two pyridoxine mutants of Neurospora reported by MITCHELL (1955). In a cross between the pyridoxine mutants, she demonstrated that pyridoxine-independent progeny could result from a process which did not show the characteristics of classical crossing over.

It is not clear whether *cys-1* and *cys-2* are involved in separate steps in the synthesis of cysteine. A study of the nutritional requirements of cysteine mutants of Neurospora (HOROWITZ 1950) suggests the following pathway of cysteine synthesis: . . . . sulfate . . . . sulfite . . . . thiosulfate . . . . (sulfide) . . . . cysteine. Both *cys-1* and *cys-2* can utilize thiosulfate (or subsequent products) for growth but cannot utilize sulfite or sulfate. However, the two mutants do show some differences in growth response. The response to thiosulfate of *cys-1* is much weaker than is that of *cys-2*. *Cys-1* strains on cysteine grow poorly at 25° but well at 35°; the growth at 25° is enhanced by the addition of tyrosine (HOROWITZ and SHEN 1952). *Cys-2* grows well on cysteine at either temperature and shows no response to tyrosine. FLING (unpublished) has found that some reisolates of *cys-1* show no response to tyrosine, and it is not yet known what part modifiers may play in the growth characteristics.

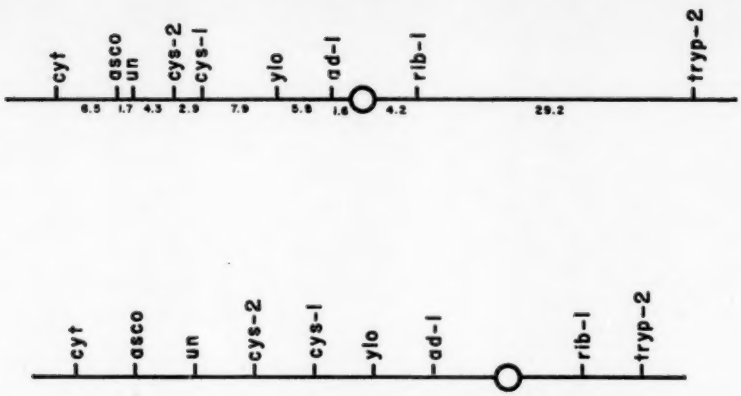

FIGURE 6.—Described in text.

The arrangement of the nine mutants with respect to each other and to the centromere is shown in figure 6. In the upper map the lengths of the intervals between neighboring loci are determined by the highest recombination frequencies observed for these intervals in the crosses reported in this paper. The author places no significance on the absolute numbers involved, and even the relative lengths of the intervals should be considered as only the crudest kind of guides for further studies. With our present limited knowledge of the factors influencing crossing over frequency in Neurospora, the less presumptious map at the bottom of figure 6 is perhaps the most appropriate representation of the findings reported here.

SUMMARY

An ascospore mutant of *Neurospora crassa* has been described and shown to be in linkage group VI. Crosses of the ascospore mutant to eight linked mutants have been analyzed in order to determine the positions of these loci relative to the ascospore mutant locus and to the centromere.

Three and four point crosses have been studied to establish the order of arrangement of neighboring mutant loci.

Some factors influencing crossing over frequency in Neurospora have been discussed, and it has been concluded that absolute distances on crossover maps can have only limited significance in our present state of knowledge.

An effect of temperature of incubation on frequency of recombinants recovered from a cross has been described and has been utilized in determining the position of a mutant locus closely linked to the centromere.

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ALLELISM AND COMPARATIVE GENETICS OF FERTILITY RESTORATION OF CYTOPLASMICALLY POLLEN STERILE MAIZE

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Received January 20, 1956

THE GENETICS of restoration of pollen fertility to cytoplasmically pollen sterile maize has been the subject of widespread investigation for the past five or six years, although to date not much of the accumulated genetic data has been published. Data presented by JONES (1951), JONES and MANGELSDORF (1951) and EDWARDSON (1955) indicate that complete fertility restoration in T cytoplasm (explained below) is determined by a single dominant gene. However, unpublished data of some of the other workers in this field have indicated that in some cases other types of gene action might operate. At times conflicting reports have been given for the same fertility restorer (FR) inbred line. In the course of a series of investigations originally designed only to compare the effectiveness of fertility restoration of several FR lines, a few principles have been demonstrated which serve to explain some of the divergent results. Additionally, these experiments have indicated the probable genotypes (with regard to fertility restoration) of several FR and sterilizable (NR) lines, to an extent that it is worth while to present them as hypotheses, acknowledging that complete genetic proof of the hypotheses is not yet at hand.

RHOADES described an instance of cytoplasmically inherited pollen sterility in maize in 1931 and 1933, but little additional study was made of this phenomenon until JONES and EVERETT (1949) outlined a method which the senior author, in cooperation with DR. P. C. MANGELSDORF, had developed to utilize cytoplasmic pollen sterility for production of hybrid seed corn without detasseling. In 1950 JONES stated that some genotypes (NR) were sterile when in "sterile" cytoplasm, and others (FR) would interact with "sterile" cytoplasm in such a way as to produce normal pollen fertile plants; i.e., interaction of both nuclear and cytoplasmic factors is responsible for the cytoplasmic pollen sterile condition. The F_1 of NR $\times$ FR, in "sterile" cytoplasm, was pollen fertile. Segregation of the nuclear factors was demonstrated. JOSEPHSON and JENKINS in 1948 had shown that the interaction of nuclear and cytoplasmic factors is responsible for a type of cytoplasmic pollen sterility discovered in the inbred 33-16. JONES and MANGELSDORF in 1951 stated that many inbreds are still segregating for nuclear factors for fertility restoration. They described 3 different sources of cytoplasmic sterility, designated them "S", "T" and "B", and stated that some genotypes were sterile in one but would restore pollen fertility (more or less) to another source of cytoplasm; in other words all types of sterility-inducing cytoplasm are not necessarily identical. Backcross and F_2 data published by them, and by JONES (1951), indicated that in T cytoplasm fertility restoration by the inbred Ky21 is due to a single dominant gene and that

sterilizability of the inbreds C106 and Ky39 is due to a single recessive gene. ROGERS and EDWARDSON in 1952 stated that inbreds K55, Tx127c and TxGJ39 produced completely fertile F_1 hybrids when crossed to the pollen sterile inbred Tx203Ms (T cytoplasm). EDWARDSON (1955) after classifying 23 F_2 populations and 13 backcross populations from crosses of cytoplasmic pollen sterile × Latin American open pollinated varieties concluded that fertility restoration appeared to be due to the action of a single dominant gene, although he mentioned the possibility that in some cases it could be due to 2 factors with dominant and recessive epistasis. C106 in T cytoplasm was used as pollen sterile tester.

METHODS AND MATERIALS

Method of classifying plants for tassel fertility

It should be emphasized that all data presented herein are based on observations of the anthers of living plants during their flowering period. At the time of flowering, plants were examined every other day. Exserted anthers were classified into 3 groups: (1) Plump, normal appearing anthers with a large pore through which pollen obviously had been shed were called "fertile anthers". Random checks (made throughout the flowering period each season) with a hand microscope and with iodine stain and a compound microscope indicated that in every instance 90 to 100% of the pollen shed by these anthers looked viable (plump, filled with purple-staining granules). Nevertheless, it should be kept in mind that in this report all discussion of the "pollen fertility" of various plants is based primarily on the appearance of their anthers rather than on the appearance of the pollen in the anthers. (2) Anthers which had a pore and shed some pollen, but which were shrunken in some places and plump in others, and in general looked abnormal, were called "partially fertile anthers". Spot checks with the microscope showed that 10 to 90% of the pollen in these anthers looked viable, and that the most abnormal anthers usually had the lowest percentages of viable-appearing pollen. (3) Exserted anthers which had no pore and thus could not shed pollen and which were extremely shrunken throughout their length were called "sterile anthers". Usually, all pollen in these anthers looked sterile, although small numbers of apparently fertile pollen grains occasionally were found. All anthers which remained within the glumes (non-exserted) also were classified as "sterile anthers". Microscopic examination showed that these anthers virtually never contained viable-appearing pollen grains.

Using this anther classification as a basis, each tassel was put into one of 5 major classes, as follows:

I. No anthers exserted.

II. Anthers exserted, but all (or very nearly all) anthers are sterile.

III. Anthers exserted and in any of the following combinations: (1) some sterile, some partially fertile and some fertile (on a single tassel), or (2) only sterile and partially fertile anthers, or (3) only partially fertile and fertile anthers, or (4) only partially fertile anthers. Tassel as a whole tends to be more nearly sterile than fertile.

IV. Similar to III, but tassel as a whole tends to be more nearly fertile.

V. All (or very nearly all) exserted anthers are fertile.

Tassels falling into one of the last 4 classes were further classified (by means of rough estimates) into one of 3 sub-classes, as follows:

A. Less than $\frac{1}{3}$ of the total number of anthers on the tassel exserted.

B. $\frac{1}{3}$ to $\frac{2}{3}$ of the anthers exserted.

C. $\frac{2}{3}$ or more anthers exserted, except in the case of Class V. Sub-class C of Class V included only those plants in which virtually all of the anthers were exserted, and all other Class V plants having more than $\frac{1}{3}$ exserted anthers were placed in sub-class B. Class VC, therefore, contained all of the plants with fully fertile tassels.

The repeatability of tassel classifications according to the standards outlined above was tested by having several persons classify the same group of plants in a segregating population. It was found that in most cases a given plant was given the same (or, at the least, a closely related) classification by all persons. The most difficult classifications were those which required one to distinguish between classes III and IV.

Lines studied and types of crosses made

The experiments to be discussed here may be divided into 4 groups: (1) tests for allelic relationships among the genes responsible for the fertility restoration properties of 5 FR inbred lines; (2) a series of backcrosses involving each of these FR lines and using the NR inbred WF9 (usually in T cytoplasm) as the recurrent parent of each backcross; (3) similar to section 2, involving Ky21 (FR inbred) with NR inbreds C106, K77, K4 and SK2 (in T cytoplasm on one side and in N cytoplasm on the other side of the cross) as recurrent parents, and BH2 (FR inbred) with C106 and K4 (in both T and N cytoplasms) as recurrent parents; (4) 3-way testcrosses, involving BH2 and Ky21, with C106 and WF9 (in both T and N cytoplasms) used as sterilizable testers.

The lines studied, their characteristics with regard to cytoplasmic sterility and the institutions from which they were obtained are shown in the following summary. FR lines restore fertility; NR (non-restorer) lines are sterilizable. T cytoplasm is "sterile"; N is "normal".

T cytoplasm is indicated according to JONES' notation, by means of a superscript after the inbred pedigree. The absence of a superscript is understood to mean that the inbred genotype is in "normal" cytoplasm. T cytoplasm will be designated also as "Cyt^T", in contrast to normal cytoplasm, "Cyt^N" during discussion of general genetic hypotheses.

F_1 hybrids were made in the 10 possible combinations of the 5 FR lines. The F_1 hybrids then were crossed to $WF9^T$ and the resulting 3-way crosses were grown and classified. $WF9^T$ used as female parent for these crosses was from a sample of composited ears. A single F_1 ear (FR $\times$ FR) was used to provide the F_1 plants for each 3-way cross. Three ear progenies per 3-way cross were grown.

For the second series of tests, $WF9^T$ was backcrossed as female to each of the five possible F_1 hybrids of $WF9^T \times$ FR. In several instances an equivalent of the re-

Inbred	Source of seed	Sterilizability	Cytoplasm
$C106^T$	Conn. Agric. Exp. Sta.	NR	T
$K4^T$	Conn. Agric. Exp. Sta.	NR	T
$WF9^T$	Conn. Agric. Exp. Sta.	NR	T
C106	Conn. Agric. Exp. Sta.	NR	N
K4	Conn. Agric. Exp. Sta.	NR	N
WF9	Conn. Agric. Exp. Sta.	NR	N
$K77^T$	Pioneer Hi-Bred Corn Co.	NR	T
$SK2^T$	Pioneer Hi-Bred Corn Co.	NR	T
K77	Pioneer Hi-Bred Corn Co.	NR	N
SK2	Pioneer Hi-Bred Corn Co.	NR	N
BH2	Pioneer Hi-Bred Corn Co.	FR	N
F5DD1	Pioneer Hi-Bred Corn Co.	FR	N
WG3	Pioneer Hi-Bred Corn Co.	FR	N
Ky21	Kentucky Agric. Exp. Sta.	FR	N
K55	Kansas Agric. Exp. Sta.	FR	N

ciprocal cross also was made, using WF9 in normal cytoplasm as pollen parent. In either instance, therefore, the backcross had T cytoplasm. Each F_1, and each backcross, was made with bulked seed (a mixture of seed from several ears of the inbred or F_1 used to make the cross) but the final backcross ears were kept separate, and were planted as individual ear progenies. Usually three progenies per backcross were planted, each progeny replicated three times. Thus, in a typical backcross, the three progenies were derived from three FR plants and six $WF9^T$ plants (or three $WF9^T$ and three WF9 plants, in the case of the "reciprocal" crosses). It is probable that the three FR plants were derived from three different parent ears and that the $WF9^T$ (and WF9) plants also were from separate parent ears.

For the third series of tests, the F_1 hybrids $C106^T \times Ky21$, $K4^T \times Ky21$, $K77^T \times Ky21$, $SK2^T \times Ky21$, $C106^T \times BH2$ and $K4^T \times BH2$ were backcrossed, each to the normal cytoplasm form of its NR inbred. In all cases the F_1 was used as female. The backcrosses involving K77 and SK2 were made with bulked seed in the same way as described for the WF9 backcrosses. Each of the other backcrosses was made with plants from a single ear progeny of the F_1. Thus, each set of these backcross progenies tested one NR^T plant of the recurrent parent, one FR plant and several NR plants of the recurrent parent. Three ear progenies of each backcross of this third series of tests were grown in each season. Each progeny was replicated three times.

The crosses of the fourth series were made by crossing an F_1 hybrid ($NR^T \times$ FR) to a different NR inbred. The 3-way cross $(C106^T \times Ky21)WF9$ was made with plants of the same F_1 progeny used to make the backcross $(C106^T \times Ky21)$-C106. The 3-way $(C106^T \times BH2)WF9$ was made with plants of the same F_1 progeny used to make the backcross $(C106^T \times BH2)C106$. Thus, in each instance a single FR plant and a single $C106^T$ plant have been tested in 2 types of cross. The

3-way ($WF9^T \times$ BH2)C106 was made from the same bulked sample of F_1 seed that was used to make ($WF9^T \times$ BH2)WF9, and so did not test the same BH2 and $WF9^T$ plants (necessarily) that were used to make the backcross, but it did test a series of plants drawn from the same samples that had furnished the BH2 and $WF9^T$ plants tested in the WF9 backcross. Two to three ear progenies per 3-way cross were grown, three replications per progeny.

No FR or partially FR plants (with respect to T cytoplasm) have ever been found by me in any of the above mentioned NR lines. Each F_1 plant that was used to make a backcross or a 3-way cross was completely fertile (tassel classification VC) at the time of crossing. Samples of the F_1 hybrids used to make each backcross were grown next to the corresponding backcross and both were classified at the same time. All populations discussed herein were grown at Johnston, Iowa during the seasons of 1953, 1954 or 1955 except for one backcross: ($C106^T \times$ BH2)C106, which also was grown near Homestead, Florida, during the winter of 1954–5.

RESULTS

Allelism testcrosses

Almost all of the plants of each allelism testcross were fully fertile, as is shown in table 1. Every cross, however, produced a few partially fertile plants, usually of tassel classification IVC or VB. In $WF9^T$(WG3 $\times$ BH2) and in $WF9^T$(WG3 $\times$ Ky21), three and one plants, respectively, were recorded as sterile. When small progenies of selfed VC plants from each progeny of each test cross were grown in 1955, about 30 to 45% of the plants in every progeny were sterile indicating that in no case was fertility of the testcross due to the absence of T cytoplasm.

Backcrosses involving five fertility restorer lines with WF9 as recurrent parent

These backcross progenies segregated in ratios of about one fertile: three sterile plants, as is shown in table 2. Most plants were either fully fertile or completely sterile. The backcross progenies involving F5DD1 had more partially fertile and fewer sterile plants than did any of the other backcrosses. Various chi square computations (tables 3, 4 and 5) were made after compositing classifications I through IIC (sterile) and IIIA through VC (fertile). Calculations of sampling chi square (table 3) showed no significant differences among replications of any backcross except $WF9^T$($WF9^T \times$ Ky21), indicating that at a given location classification results usually are repeatable. Most backcross progenies gave a satisfactory fit to a 1:3 ratio, as is shown in table 4. All backcrosses involving F5DD1 deviated significantly ($P < .05$) from 1:3 but when the F5DD1 data were recalculated after putting all partially fertile plants into the "sterile" class a reasonably good fit to a 1:3 ratio was obtained for all progenies. Calculations of heterogeneity chi square indicate that: (1) all progenies of the WF9 backcrosses (except those involving F5DD1) are reasonably similar with respect to goodness of fit to a 1:3 ratio and as a population the chances are fairly good that they are segregating in a ratio of 1:3 (table 5A); (2) segregation ratios of "reciprocal" backcrosses did not differ

TABLE 1

Allelism test crosses: WF9 ^{T} crossed to F_1 hybrids of 5 fertility restorer lines

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V		
				A	B	C	A	B	C	A	B	C	A	B	C	
WF9^T(BH2 × F5DD1)	1	1954												1	34	35
	2	1954													32	32
	3	1954													33	33
WF9^T(BH2 × Ky21)	1	1954												3	30	33
	2	1954													33	33
	3	1954													37	37
WF9^T(WG3 × BH2)	1	1954													34	34
	2	1954	3									1	2		29	35
	3	1954													31	31
WF9^T(K55 × BH2)	1	1954													35	35
	2	1954								1	1			1	33	36
	3	1954											2		31	33
WF9^T(F5DD1 × Ky21)	1	1954													34	34
	2	1954												1	30	31
	3	1954												1	33	34
WF9^T(F5DD1 × WG3)	1	1954													36	36
	2	1954												1	35	36
	3	1954													31	31
WF9^T(K55 × F5DD1)	1	1954											2	2	30	34
	2	1954												2	32	34
	3	1954													38	38
WF9^T(WG3 × Ky21)	1	1954	1									4		3	24	32
	2	1954										1			33	34
	3	1954										3		2	31	36
WF9^T(Ky21 × K55)	1	1954										3		3	27	33
	2	1954													32	32
	3	1954													34	34
WF9^T(WG3 × K55)	1	1954											1		29	30
	2	1954										2			30	32
	3	1954										2		1	30	33

significantly (table 5B); and (3) segregation ratios of the same backcross in 2 different years did not differ significantly (table 5C). The BH2 backcrosses compared in table 5C are not strictly comparable since the progenies grown in 1955 were not from remnant seed of the progenies grown in 1954.

TABLE 2

Backcrosses involving five fertility restorer lines; WF9 recurrent parent

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V		
				A	B	C	A	B	C	A	B	C	A	B	C	
WF9^T(WF9^T × BH2)	315-5241-1	1954	116	1						2	2	2	1	1	36	161
	315-5241-2	1954	115								1			3	51	170
	315-5241-3	1954	227		1					2	2		1		76	309
	315-5241-4	1955	77								1				16	94
	315-5241-5	1955	70							1	1	1			18	91
	315-5241-6	1955	71				1					2			30	104
WF9^T(WF9^T × F5DD1)	G301024-7	1953	119				1		1	4	1	3	1	3	56	189
	G301024-8	1953	75				3			3	1	4	3	1	30	120
(WF9^T × F5DD1) WF9	G301050-6	1953	53							3		1	5		27	89
WF9^T(WF9^T × Ky21)	315-5243-1	1954	53									1			12	66
	315-5243-2	1954	161											2	67	230
	315-5243-1	1955	14									2			1	17
	315-5243-2	1955	13									2			6	21
(WF9^T × Ky21) WF9	425203-1	1955	76									1			12	89
	425203-2	1955	70									6			23	99
	425203-3	1955	73									3			21	97
WF9^T(WF9^T × K55)	315-5253-1	1954	51		1										16	68
	315-5253-2	1954	55												15	70
	315-5253-3	1954	50	1										1	13	65
	315-5253-4	1954	53				2								14	69
	315-5253-5	1954	47				1	1		1	1				12	63
	315-5253-6	1954	47		1				1						22	71
	315-5253-7	1954	52											2	17	71
	315-5253-8	1954	50												16	66
	315-5253-9	1954	50				2								16	68
WF9^T(WF9^T × WG3)	G301018-1	1953	75							1					20	96
(WF9^T × WG3) WF9	G302060-4	1953	34												12	46
	G302060-5	1953	20												8	28

Ky21 backcrossed with four different recurrent parents and BH2 backcrossed with two different recurrent parents

About one half of the plants in the backcross progenies involving C106, K4, K77 and SK2 were sterile (table 6). These results are in sharp contrast to the 1:3 ratios obtained for backcrosses involving WF9 (table 2). An additional difference was that many pollen shedding plants of the backcrosses involving C106, K4 and K77

TABLE 3

Chi square test for uniformity of sampling; backcrosses of five FR lines with WF9 as recurrent parent

Pedigree	Year	Number of replications	Number of classes*	DF	Sampling χ^2	*P*
WF9^T(WF9^T × BH2)	1954	3	2	3	.869	.83
	1955	3	2	3	3.157	.38
(WF9^T × Ky21) WF9	1955	3	2	3	1.019	.80
WF9^T(WF9^T × Ky21)	1954	2	2	2	12.880	<.01
WF9^T(WF9^T × F5DD1)	1953	3	2	3	1.887	.60
(WF9^T × F5DD1) WF9	1953	3	2	3	4.726	.19
WF9^T(WF9^T × K55)	1954	2	2	2	.026	.99
(WF9^T × WG3) WF9	1953	2	2	2	.002	>.99

* Two classes are: (1) tassel classifications I through IIC, "sterile", and (2) tassel classifications IIIA through VC, "pollen shedding."

were not completely fertile. They usually were classified as IVC. (C106^T × Ky21)-C106, however, had large numbers of IIIA, IIIB and IIIC plants. In all backcrosses class IV tassels were easily distinguished from class V tassels, and in (C106^T × Ky21)C106 the class III tassels usually were easily distinguished from class IV tassels. Occasional intergrades occurred in all backcrosses, however. Sampling chi squares (table 7A) show that with one exception there were no significant differences among replications of a backcross. Most backcross progenies gave a satisfactory fit to a 1:1 ratio as is shown in table 8. Tassel classifications were combined into "sterile" and "pollen shedding" classes prior to this computation, as was done for the WF9 backcrosses. The ratios of three backcross progenies deviate significantly ($P < .05$) from a 1:1 ratio, and two or three other progenies show a rather poor fit. With two exceptions, progenies within a backcross were not significantly heterogeneous with respect to goodness of fit to a 1:1 ratio (table 9).

It is apparent in table 6 that although progenies within a given backcross usually have similar percentages of sterile plants, they often differ from each other with respect to proportions of partially fertile:fertile plants. When the sampling chi square method is used to measure this variability (progenies, instead of replications, are treated as samples) it can be shown (table 7B) in several instances that progenies of a backcross are significantly heterogeneous ($P < .05$). In most instances the heterogeneity is due to differences in proportions of partially fertile:fertile plants. The fact that each backcross which had a high sampling chi square for progenies nevertheless had a low sampling chi square for replications is further evidence that progeny differences were based on genetic differences, for they were repeated in the same manner from replication to replication. It also shows that the classification system was reasonably reliable. One can conceive that if all differences in classification were due to random choice of a classification for each tassel, the choices could be randomized in about the same fashion from replication to replication and thus give a low sampling chi square for replications. However, if such were true these choices also should have been randomized within the progenies included in each replication, resulting in a low sampling chi square for progenies as well.

TABLE 4

Chi square tests for goodness of fit to 1:3 ratio; backcrosses of five FR lines, with WF9 as recurrent parent

Pedigree	Year	Ear number	Total plants	Sterile plants		χ^2 for 1:3 ratio*	*P*
				Obs.	Exp.		
WF9^T(WF9^T × BH2)	1954	315-5241-1	161	117	120.75	.465	.50
		315-5241-2	170	115	127.50	4.901	.03
		315-5241-3	309	228	231.75	.244	.64
	1955	315-5241-4	94	77	70.50	2.397	.13
		315-5241-5	91	70	68.25	.180	.68
		315-5241-6	104	71	78.00	2.513	.12
WF9^T(WF9^T × F5DD1)	1953	G301024-7	189	119	141.75	14.605	<.01
				133†	141.75	2.160	.16
		G201024-8	120	75	90.00	10.000	<.01
				90†	90.00	0.000	>.99
(WF9^T × F5DD1) WF9	1953	G301050-6	89	43	66.75	11.329	<.01
				62†	66.75	1.352	.25
WF9^T(WF9^T × Ky21)	1954	315-5243-1	66	53	49.50	.989	.33
		315-5243-2	230	161	172.50	3.057	.08
	1955	315-5243-1	17	14	12.75	.491	.49
		315-5243-2	21	13	15.75	1.921	.17
(WF9^T × Ky21) WF9	1955	425203-1	89	76	66.75	5.127	.03
		425203-2	99	70	74.25	.973	.33
		425203-3	97	73	72.25	.004	.95
WF9^T(WF9^T × K55)	1954	315-5253-1	68	52	51.00	.079	.78
		315-5253-2	70	55	52.50	.576	.49
		315-5253-3	65	51	48.75	.416	.53
		315-5253-4	69	53	51.75	.121	.73
		315-5253-5	63	47	47.25	.005	.94
		315-5253-6	71	48	53.25	2.071	.16
		315-5253-7	71	52	53.25	.117	.74
		315-5253-8	66	50	49.50	.020	.89
		315-5253-9	68	50	51.00	.079	.78
WF9^T(WF9^T × WG3)	1953	G301018-1	96	75	72.00	.500	.49
(WF9^T × WG3) WF9	1953	G302060-4	46	34	34.50	.029	.87
		G302060-5	28	20	21.00	.191	.67

* Calculated for two classes as follows, except where indicated otherwise: (1) "sterile", I through IIC, and (2) "pollen shedding" IIIA through VC.

† "Sterile" class includes classes I through VB. Fertile class is VC only.

F_1 hybrids of cytoplasmic sterile lines × fertility restorer lines

When samples of the F_1 hybrids used to make each backcross were grown beside the corresponding backcross populations, it was found that a few plants in each F_1 were classified as incompletely fertile (usually IVC), as shown in table 10. C106^T ×

TABLE 5

Heterogeneity chi squares for backcrosses tested against 1:3 ratio

A. Among progenies of a backcross in one year

Pedigree	Year	Number of progenies	DF	Heterogeneity χ^2	*P*
WF9^T(WF9^T × BH2)	1954	3	2	2.277	.34
	1955	3	2	5.061	.08
WF9^T(WF9^T × F5DD1)	1953	2	1	.839*	.38
WF9^T(WF9^T × Ky21)	1954	2	1	2.930	.09
	1955	2	1	2.096	.16
(WF9^T × Ky21) WF9	1955	3	2	5.588	.06
WF9^T(WF9^T × K55)	1954	9	8	3.383	.91
(WF9^T × WG3)WF9^T	1953	2	1	.325	.58
All progenies except F5DD1 crosses		25			
Total			25	27.376	.34
Pooled			1	1.083	.30
Interaction			24	26.293	.34

B. Between "reciprocal" backcrosses

Pedigree	Year	DF	Heterogeneity χ^2	*P*
WF9^T(WF9^T × F5DD1) vs. (WF9^T × F5DD1) WF9	1953	1	.230	.65
WF9^T(WF9^T × Ky21) vs. (WF9^T × Ky21) WF9	1955	1	.600	.45
WF9^T(WF9^T × WG3) vs. (WF9^T × WG3) WF9	1953	1	.592	.46

C. Between years

Pedigree	Years	DF	Heterogeneity χ^2	*P*
WF9^T(WF9^T × BH2)	1954 vs. 1955	1	1.345	.25
WF9^T(WF9^T × Ky21)	1954 vs. 1955	1	.337	.58

* "Sterile" class includes tassel classifications I through VB.

Ky21 had an especially high proportion of such plants. Anthers of even the VC plants in this F_1 did not look quite as fertile as those of other F_1 hybrids. The IVC plants were not sharply distinct from VC; rather, they represented a point in a continuous series at which one arbitrarily decided he must call the plant IVC, instead of VC. The IVC tassels in other F_1 hybrids likewise were not sharply distinct from VC; that is, they had a few too many partially fertile anthers to be called VC, but did not have nearly as many partially fertile anthers as the majority of IVC plants

TABLE 6

Backcrosses involving Ky21 and BH2 with C106, K4, K77 and SK2 as recurrent parents

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V		
				A	B	C	A	B	C	A	B	C	A	B	C	
(C106^T × Ky21)	F406049-1	1954	98	1			4	5	15	2	5	67			10	207
C106	F406049-2	1954	101				8	8	13	4	6	47			3	190
	F406049-3	1954	118				5	3	13	1	3	42			7	192
	F406049-4	1955	56				8	4	15	2		19				104
	F406049-5	1955	31				6	1	15			16			14	83
	F406049-6	1955	48				6	1	19	1		20			3	98
(K4^T × Ky21)	F407038-1	1954	95					1	10			51			34	191
K4	F407038-2	1954	114						7	3		53	1		30	208
	F407038-3	1954	93				3		8	9	1	45	2	2	34	197
(K77^T × Ky21)	F506059-10	1955	40						5	1		45			7	98
K77	F506059-11	1955	44								1	4			41	90
	F506059-12	1955	45									9			36	90
(SK2^T × Ky21)	F506062-11	1955	47												48	95
SK2	F506062-12	1955	44									1			43	88
	F506062-13	1955	45											1	53	99
(C106^T × BH2)	F405039-1	1954	102						4		2	49	1	1	38	197
C106	F405039-2	1954	106					2	4		1	56	1		36	206
	F405039-3	1954	104				1		5	1	4	52		1	34	202
	F405039-5	1955	52				1	1	13	2		23			7	99
	F405039-6	1955	56					1	4			19	1		17	98
	F405039-7	1955	41				1	1	3			23			34	103
(K4^T × BH2)	F407036-1	1954	100	1			1		5	1		45		1	53	207
K4	F407036-2	1954	102						20	1		61	1		30	215
	F407036-3	1954	103	1			1		10		3	53			27	198

TABLE 7

Chi square tests for uniformity of sampling, comparing (a) replications and (b) progenies of backcrosses involving Ky21 and BH2, with C106, K4, K77 and SK2 as recurrent parents

Pedigree	Year	Number classes	A. Comparing replications				B. Comparing progenies			
			Number reps.	DF	Sampling χ^2	P	Number progenies	DF	Sampling χ^2	P
(C106^T × Ky21) C106	1954	4***	3	9	9.849	.46	3	9	12.882	.17
	1955	4	3	9	5.986	.74	3	9	26.975	< .01
(K4^T × Ky21) K4	1954	3**	3	6	30.118	< .01	3	6	2.727	.84
(K77^T × Ky21) K77	1955	3	3	6	2.836	.85	3	6	81.710	< .01
(SK2^T × KY21) SK2	1955	2*	3	3	.184	.98	3	3	.474	.92
(C106^T × BH2) C106	1954	3	3	6	3.486	.75	3	6	.636	> .99
	1955	3	3	6	.899	.99	3	6	25.413	< .01
(K4^T × BH2) K4	1954	3	3	6	3.972	.67	3	6	16.938	< .01

* Two classes are: (1) "sterile", I through IIC; (2) "pollen shedding", IIIA through VC.

** Three classes are: (1) "sterile", I through IIC; (2) "partially fertile," IIIA through VB, (3) "fertile," VC.

*** Four classes are: (1) "sterile," I through IIC; (2) "low grade partially fertile," IIIA through IIIC; (3) "high grade partially fertile" IVA through VB; (4) "fertile" VC.

TABLE 8

Chi square tests for goodness of fit to 1:1 ratio; backcrosses involving Ky21 and BH2, with C106, K4, K77 and SK2 as recurrent parents

Pedigree	Year	Ear number	No. of plants	Sterile plants		x^2 for 1:1 ratio*	*P*
				Obs.	Exp.		
(C106^T × Ky21) C106	1954	F406049-1	207	99	103.50	.392	.54
		F406049-2	190	101	95.00	.758	.40
		F406049-3	192	118	96.00	10.084	< .01
	1955	F406049-4	104	56	52.00	.616	.45
		F406049-5	83	31	41.50	5.314	.02
		F406049-6	98	48	49.00	.040	.85
(K4^T × Ky21) K4	1954	F407038-1	191	95	95.50	.006	.94
		F407038-2	208	114	104.00	1.924	.17
		F407038-3	197	93	98.50	.614	.45
(K77^T × Ky21) K77	1955	F506059-11	98	40	49.00	3.306	.08
		F506059-12	90	44	45.00	.004	.84
		F506059-13	90	45	45.00	.000	> .99
(SK2^T × Ky21) SK2	1955	F506062-11	95	47	47.50	.010	.93
		F506062-12	88	44	44.00	.000	> .99
		F506062-13	99	45	49.50	.818	.38
(C106^T × BH2) C106	1954	F405039-1	197	102	98.50	.228	.65
		F405039-2	206	106	103.00	.174	.68
		F405039-3	202	104	101.00	.178	.68
	1955	F405039-5	99	52	49.50	.252	.63
		F405039-6	98	56	49.00	2.000	.17
		F405039-7	103	41	51.50	4.282	.04
(K4^T × BH2) K4	1954	F407036-1	207	101	103.50	.120	.73
		F407036-2	215	102	107.50	.562	.47
		F407036-3	198	104	99.00	.506	.48

* Calculated for two classes: (1) "sterile", I through IIC; (2) "pollen shedding," IIIA through VC.

TABLE 9

Heterogeneity chi squares for backcrosses tested against 1:1 ratio

Pedigree	Number of progenies	Year	DF	Heterogeneity x^2	*P*
(C106^T × Ky21) C106	3	1954	2	7.484	.02
		1955	2	5.180	.08
(K4^T × Ky21) K4	3	1954	2	2.436	.30
(K77^T × Ky21) K77	3	1955	2	1.912	.40
(SK2^T × Ky21) SK2	3	1955	2	.474	.79
(C106^T × BH2) C106	3	1954	2	.004	> .99
	3	1955	2	6.520	.04
(K4^T × BH2) K4	3	1954	2	1.130	.58

in their corresponding backcrosses. $K4^T \times$ Ky21 and $K77^T \times$ Ky21 had one and two sterile plants, respectively. These plants were phenotypically similar to others in the F_1 and did not appear to be outcrosses.

3-way crosses involving two NR lines

Ratios of approximately one sterile to one pollen shedding plant were obtained for all progenies of $(C106^T \times$ Ky21)WF9, $(C106^T \times$ BH2)WF9, and $(WF9^T \times$

TABLE 10

F_1 hybrids of cytoplasmically pollen sterile lines by fertility restorer lines, grown next to their corresponding backcross progenies

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V			
				A	B	C	A	B	C	A	B	C	A	B	C	
$WF9^T \times$ BH2	bulk	1954										65			65	65
		1955										5			64	69
$WF9^T \times$ F5DD1	bulk	1953												2	176	178
$WF9^T \times$ Ky21	bulk	1954													71	71
		1955										2			44	46
$WF9^T \times$ K55	bulk	1954										1			66	67
$WF9^T \times$ WG3	bulk	1953										5		1	65	71
		1954													31	31
$C106^T \times$ Ky21	1	1954										11	1	1	22	35
		1955										19			29	48
$K4^T \times$ Ky21	6	1954	1									4	1		62	68
$K77^T \times$ Ky21	bulk	1955	2									5			41	48
$SK2^T \times$ Ky21	bulk	1955													41	41
$C106^T \times$ BH2	3	1954										2		4	66	72
		1955										2			74	76
$K4^T \times$ BH2	1	1954													69	69

TABLE 11

3-Way crosses involving Ky21 and BH2

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V			
				A	B	C	A	B	C	A	B	C	A	B	C	
$(C106^T \times$ Ky21) WF9	F406047-1	1955	63									6			40	109
	F406047-2	1955	52									2			50	104
	F406047-3	1955	54									3			48	105
$(C106^T \times$ BH2) WF9	F405041-1	1955	40				1		1						63	105
	F405041-2	1955	46					1				1			53	101
	F405041-3	1955	49				1	1				1			44	96
$(WF9^T \times$ BH2) C106	F405043-2	1955	49				2					1		1	38	91
	F405043-3	1955	38						1			3			50	92

TABLE 12

Chi square tests for goodness of fit to 1:1 ratio; 3-way crosses involving Ky21 and BH2

Pedigree	Year	Ear number	Total plants	Sterile plants		χ^2 for 1:1 ratio*	*P*
				Obs.	Exp.		
(C106^T × Ky21) WF9	1955	F406047-1	109	63	54.50	2.652	.11
		F406047-2	104	52	52.00	0.000	>.99
		F406047-3	105	54	52.50	.086	.77
(C106^T × BH2) WF9	1955	F405041-1	105	40	52.50	5.952	.02
		F405041-2	101	46	50.50	.802	.39
		F405041-3	96	49	48.00	.042	.85
(WF9^T × BH2) C106	1955	F405043-2	91	49	45.50	.538	.47
		F405043-3	92	38	46.00	2.782	.10

* Calculated for two classes: (1) "sterile", I through IIC; (2) "pollen shedding", IIIA through VC.

TABLE 13

Heterogeneity chi squares for 3-way crosses tested against 1:1 ratio

Pedigree	Year	Number progenies	DF	Heterogeneity χ^2	*P*
(C106^T × Ky21) WF9	1955	3	2	1.480	.48
(C106^T × BH2) WF9	1955	3	2	3.406	.19
(WF9^T × BH2) C106	1955	2	1	2.878	.09

TABLE 14

(C106^T × BH2) C106 grown near Homestead, Florida during winter of 1954–5

Ear number	Number of plants per tassel classification												Total	χ^2 for 1:1 ratio	*P*
	I	II			III			IV			V				
		A	B	C	A	B	C	A	B	C	A	B	C			
F405039-1	57												76	133	2.714	.10
F405039-2	107												94	201	.840	.38
F405039-3	46												66	112	3.572	.06

BH2)C106, as is shown in table 11. Nearly all of the pollen shedding plants were fully fertile. In all but one instance progeny ratios did not deviate significantly from 1:1 (table 12). Table 13 shows that with respect to a 1:1 ratio progenies of no 3-way cross were significantly heterogeneous.

Backcross grown in Florida

Table 14 shows that when the three ear progenies of (C106^T × BH2)C106 that had been grown in Iowa in 1954 were grown in Florida in the winter of 1954–5 all pollen shedding plants were classified as VC, despite the fact that in Iowa many of

the pollen shedding plants were only partially fertile. In both locations about the same proportions (one half) of the plants were sterile. Chi square tests for goodness of fit to a 1:1 ratio of each of the progenies grown in Florida show no significant deviations from 1:1, but two progenies have low P values, due in both cases to an excess of fertile plants.

DISCUSSION

Three general conclusions can be drawn from the summaries of data presented in the previous section. In the first place, it is apparent that all five FR lines (in so far as they were sampled) have the same alleles of the major gene or genes needed to restore pollen fertility to $WF9^T$. Otherwise, segregation among gametes produced by the F_1 plants would have resulted in the presence of several sterile plants in the test cross $WF9^T$ (FR × FR). The data in table 1 do not demonstrate, however, whether or not all of the FR lines contain all of the factors needed for complete fertility restoration. It is conceivable that the WF9 genotype may include one or more genes which act in a complementary fashion with others furnished by the FR lines. Nor do the data prove that some FR lines do not have more FR factors than do other FR lines; that is, duplicate, non-allelic FR factors could exist in some lines but not in others. Such duplicates would not be detected by the type of testcross that has been made. It may be important to note that four plants (in table 1) were recorded as being sterile. Three of them were in one progeny of $WF9^T$ (WG3 × BH2). The three progenies of this cross were grown again in 1955 and no sterile plants were seen. It is possible that the plants were recorded as sterile by accident in 1954 (lost classification tag, for instance) or that they were the result of contaminating pollen grains on the $WF9^T$ ear. It also is conceivable that they could be the result of infrequent crossovers between closely linked FR factors of BH2 and WG3, or of mutation of a dominant FR factor to the recessive state. Either hypothesis needs more supporting data, however, before it can pass from the stage of conjecture. The small numbers of partially fertile plants appearing in each 3-way cross are not too unexpected since the F_1 hybrids of $WF9^T$ × FR also had a few partially fertile plants.

The second generalization pointed to by the data is that a backcross involving a given FR line (for example, Ky21) may segregate in one fashion when a given NR line (say, WF9) is used as a recurrent parent, but may segregate in an entirely different fashion when another NR line (C106, for example) is used as the recurrent parent. Additionally, if a testcross is made which uses both NR lines (($C106^T$ × Ky21)WF9, for example) a third type of segregation may result. In other words, the assumption is false that any two lines which will sterilize in T cytoplasm are therefore isogenic with respect to all FR genes. For if this were so, all three types of crosses should give identical segregations. It follows, therefore, that any statement as to the number of genes responsible for the FR action of a given FR line is true only with respect to the sterilizable line used as a tester. A different sterilizable line might easily be recessive for additional FR factors, and therefore would reveal genes which could not have been discovered with the first tester. Thus, two investigators, each using a different sterilizable inbred as a tester, could study the

genetics of the same FR line and come to opposing conclusions as to the number and action of the FR genes in the FR line.

The third general conclusion (and also a logical consequence of conclusion number two) which can be drawn from these data is that a uniformly sterilizable line can vary from plant to plant within the line with regard to some of the genes which determine the degree of fertility of pollen-shedding plants. JONES and MANGELSDORF (1951) have pointed out that certain long-time inbred lines can be shown to have some plants which are NR and other plants which are FR. It is demonstrated here that heterogeneity for FR factors can exist even in lines in which all plants are NR. Whether such heterogeneity has arisen via mutation in an originally homozygous line, or whether the original line was heterozygous for these genes cannot be determined from the data at hand. All of the lines used in this study were phenotypically uniform inbreds, maintained by selfing, but all lines had been maintained by compositing selfed ears for a period of several generations. (C106^T × BH2)C106, all the progenies of which trace back to a single BH2 and a single C106^T plant, is one of the backcrosses which gives convincing evidence for heterogeneity within a uniformly sterilizable line. In three replications of one backcross progeny, the proportions of plants in tassel classifications III:IV:V (the three pollen-shedding classes) were (per ten plants) 3:5:2, 3:6:1, and 3:4:3 (avg. 3:5:2). In a second progeny, grown beside the first, the three replications gave ratios of 1:3:6, 1:5:4, and 1:3:6 (avg. 1:4:5). Since ratios for the three replications are very similar ($P = .85$) it follows that the large difference between ratios of the progenies ($P < .01$) almost certainly is due to a genetic difference between the progenies. If one assumes that the two parent plants of the F_1 were homozygous, then the only hereditary difference between the two progenies was that different C106 plants were used as male parent, and any difference in tassel fertility between the two progenies must have been due to genetic differences in the two C106 plants. Nevertheless, all C106 plants that I have tested were completely sterile in Cyt^T.

It is possible that either the C106^T plant or the BH2 plant which were used to make the original F_1 ear could have been heterozygous for one or more factors which modify a major factor for restoration of pollen fertility. The F_1 plants thus would have been heterozygous and would have given rise to different types of backcross progenies even if all C106 male plants had been isogenic. If the C106^T plant had been heterozygous, the original contention that a sterilizable line may be heterogeneous for FR genes would still hold, for C106 is the pollen parent used to maintain C106^T. If the BH2 plant had been heterozygous, the F_1 also should have appeared heterozygous, but as was noted earlier this was not the case, for the few F_1 plants that were classified as high grade partially fertile did not differ sharply from VC, whereas most IVC plants of the backcross progenies were distinctly different from VC. At any rate, only VC F_1 plants were used to make backcrosses.

Several backcrosses, the progenies of which were shown to be heterogeneous with respect to tassel fertility, were made with seed from bulked F_1 ears and therefore tested a different FR plant in each progeny, as well as different NR^T and NR plants ((K77^T × Ky21)K77, for example). In these cases one can say that the FR, the NR, or even both of the inbreds in the backcross may have been heterogeneous

but one cannot determine exactly which line was so. The plant and ear characteristics of all progenies within each backcross were similar, so that it is virtually certain that in no instance was variation among progenies, with respect to pollen fertility, due to a mistake in pollinating.

The experiments presented in this study were not planned to give conclusive proof of the number of factors responsible for fertility restoration; nevertheless, they do indicate definite hypotheses which can be tested more completely in the future.

All of the FR lines tested appear to have two dominant complementary factors which are needed to restore full pollen fertility to WF9^T. That is, if WF9^T × Ky21 were of the genotype[1], *AaBb*, and WF9 were *aabb*, the backcross to WF9 would give one plant in four with the genotype *AaBb*, and three plants in four with only one dominant, or with none. The *AaBb* plants would be fully fertile, others would be fully sterile (in T cytoplasm). It is possible that additional complementary FR factors could be contributed by WF9. The backcross data alone cannot demonstrate whether or not this is the case. Also, the data do not prove whether or not the postulated two dominant complementary factors were present in every one of the fertile and the partially fertile backcross plants that were more or less arbitrarily put in the same class for chi square computation. In the case of F5DD1, as a matter of fact, it would seem more likely that the partially fertile plants did not contain both of the dominant complementary factors, since a good fit to a 1:3 ratio is achieved only when partially fertile plants are thrown into the "sterile" class. It may be that F5DD1 has, in addition to the two dominant complementary factors for full fertility, a gene or genes for partial fertility. Other FR lines could have weaker forms of similar genes for partial fertility, perhaps such that the potentiality of the genes was expressed in some environments and not in others. Conversely, it is conceivable that in some seasons random environmental conditions could affect by chance some, but not all, of the plants having the necessary two dominant FR genes, so that some *AaBb* plants would be fully fertile, but others would be only partially fertile. Or, it could be that in a uniformly severe season, factors in addition to *A* and *B* would be needed to maintain full pollen fertility. If these additional factors also were segregating in the backcross, some *AaBb* plants would have the proper forms of the additional factors and would remain fertile, but others would lack them and would be only partially fertile. It seems likely that in many situations both types of effect (environmental and genetic) could occur and would interact in various ways. Progeny tests of each of the several types of backcross plants must be made, therefore, to establish their specific genotypes.

Ky21 and BH2 seem to furnish one major dominant gene the presence of which is required to restore pollen fertility to all sterilizable lines tested. In addition they apparently furnish several dominant modifier genes which also must be present for full pollen fertility, but which have no effect without the dominant form of the major FR gene. These modifier genes would be different from, and in addition to the important second complementary dominant factor which only WF9 appears to lack. Some inbreds (in so far as they have been tested) appear to have more dominant

[1] Gene designations used throughout this discussion are merely convenient symbols, and are not presented as suggested permanent designations.

modifiers than others. SK2, for instance, seems to have all of them (see table 6; most of the pollen shedding plants are fully fertile); C106 seems to have few of them (see table 6; few of the pollen shedding plants are fully fertile).

As was the case with the WF9 backcrosses one cannot be sure until progeny tests have been made that the arbitrary division of tassel classes for calculating goodness of fit to 1:1 ratios was genetically correct in all cases. It even may be that some completely sterile plants (tassel classification I) had the major dominant FR gene, but lacked dominant modifiers to such an extent that in combination with a certain environment no pollen was shed. Some of the significant deviations of backcross progenies from 1:1 ratio may be due to some reason such as this. It is interesting to note that the WF9 backcrosses, all of whose progenies were usually more uniform with respect to segregation ratios, also appeared to be much less affected by modifier genes.

WF9 and C106 appear to be alike in that both lines apparently are homozygous recessive for the same major FR gene; but C106 seems to have the dominant form of the second complementary factor which in WF9 is recessive, and WF9 seems to have the dominant modifiers which in C106 are recessive. This hypothesis is supported by the 3-way cross segregations shown in table 11, as contrasted to the backcross segregations shown in tables 2 and 6. In the most simplified form, one could diagram an explanation of this as follows:

Cyt^T *AaBbCC* × Cyt^N *aaBBcc*		Genotype	Inbred
($WF9^T$ × FR) (C106)		*aabbCC*	WF9
		aaBBcc	C106
		AABBCC	FR
Cyt^T *AaBBCc*	2 fertile		
Cyt^T *AaBbCc*			
Cyt^T *aaBBCc*	2 sterile		
Cyt^T *aaBbCc*			

It is probable that most C106 plants are recessive for more than one modifier gene (*C*) but since WF9 also would be dominant for any additional modifiers the final ratio would not be changed. If the inbred lines may be heterozygous for modifiers as was postulated earlier, it, of course, would be necessary to determine gene differences for each sub-strain. And as was the case with the hypothesis for the WF9 backcrosses, the evidence presented here does not show whether or not either of the sterilizable lines could have contributed some FR factors not supplied by the FR line.

Further evidence for the hypothesis of a major dominant FR gene assisted by modifiers is given by the segregation of ($C106^T$ × BH2)C106 when grown in Florida in the winter of 1954–5. As has been pointed out by several workers, many lines and hybrids, in Cyt^T, are more fertile when grown in Florida in the winter than when grown in the Corn Belt during the summer. When samples of the same three progenies of ($C106^T$ × BH2)C106 that had been grown in Iowa in 1954 were grown near Homestead, Florida, in 1954–5, they segregated in about a 1:1 ratio much as they had in Iowa, but in contrast to the same progenies in Iowa, no partially fertile plants were observed. All plants were either completely fertile or completely sterile. It

would appear that under the conditions of 1954–5 in Florida no modifying gene action was required. The presence, in dominant form, of only the major FR gene was adequate for development of fully fertile tassels. Florida, 1954–5, therefore, might be regarded as having provided an environment in which one was unable to recognize the presence of any genes which modify the action of the major FR gene. The Iowa summer seems to reveal a number of modifiers, perhaps more in some years than others, and one can conceive of other environments which might reveal still others. One could argue plausibly that the difference between Florida and Iowa was merely a case of incomplete penetrance (in Iowa) of a single dominant gene, were it not for the fact that different NR backgrounds give different, repeatable ratios of "penetrance" of the same FR gene. (See discussion of (C106^T × BH2)C106 and of the contrast between SK2 and C106 backcross ratios.) It is pertinent to state here that for four years in succession I have noted, in the course of routine breeding programs, a contrast in segregating populations between Florida and Johnston similar to that described for (C106^T × BH2)C106, although in general the material was not as critical as that described here, and in some years the change to full fertility (in Florida) was not as complete as that described here.

As stated earlier, Jones found that (C106^T × Ky21)C106 produced only fully fertile and completely sterile plants, in a ratio of about 1:1. These results contrast sharply with the large proportions of partially fertile plants produced by all progenies of the same backcross in my experiments. The C106^T and C106 I used were obtained from JONES and probably still average about the same, genetically, as his. Ky21 was obtained from the Kentucky Agricultural Experiment Station and may or may not have been identical with the strain used by JONES. However, in view of the results obtained in Florida, it seems most probable that differences in number of partially

TABLE 15

Chi square tests for goodness of fit to 3:5:8 ratio; backcrosses involving BH2 and Ky21, with C106 and K4 as recurrent parents

Pedigree	Year	Ear number	DF	χ^2 for 3:5:8*	*P*
(C106^T × BH2) C106	1954	F405039-1	2	.492	.78
		F405039-2	2	.268	.88
		F405039-3	2	.498	.78
	1955	F405039-5	2	9.979	<.01
		F405039-6	2	2.139	.35
		F405039-7	2	13.861	<.01
(K4^T × BH2) K4	1954	F407036-1	2	7.360	.03
		F407036-2	2	6.638	.04
		F407036-3	2	3.441	.18
(K4^T × Ky21) K4	1954	F40738-1	2	.188	.91
		F40738-2	2	3.054	.22
		F40738-3	2	1.698	.44

* Calculated for three classes: (1) "sterile", tassel classifications I through IIC; (2) "partially fertile", classifications IIIA through VB; (3) "fertile", classification VC.

fertile plants were due to differences in climate between Iowa and Connecticut. The summers of 1954 and 1955 in Iowa were unusually hot and dry.

The number of recessive modifier genes which are present in lines other than SK2 and WF9 is not surmised very easily since there is no great uniformity of the proportions of fertile to partially fertile plants from progeny to progeny within a backcross. However, several backcross progenies do approximate ratios of 3 fertile:5 partially fertile:8 sterile plants. As is shown in table 15, P values are high for some of the progenies of (C106^T × BH2)C106 and of (K4^T × Ky21)K4 when they are tested for goodness of fit to this ratio. Segregations giving this ratio could occur if four dominant genes were postulated as follows:

		Pedigree	Genotype
Cyt^T *AaCcDdEe* × Cyt^N *aaccddee*		BH2	*AACCDDEE*
(C106^T × BH2)	(C106)	C106	*aaccddee*

Cyt^T *AaCcDdEe*	3 fertile
Cyt^T *AaCcDdee*	
Cyt^T *AaCcddEe*	
Cyt^T *AaCcddee*	5 partially fertile
Cyt^T *AaccDdEe*	
Cyt^T *AaccDdee*	
Cyt^T *AaccddEe*	
Cyt^T *Aaccddee*	
Cyt^T *aaCcDdEe*	8 sterile
Cyt^T *aaCcDdee*	
Cyt^T *aaCcddEe*	
Cyt^T *aaCcddee*	
Cyt^T *aaccDdEe*	
Cyt^T *aaccDdee*	
Cyt^T *aaccddEe*	
Cyt^T *aaccddee*	

This scheme would postulate that a single dominant gene (A) must be present in order to have any pollen fertility, and a second (C) plus one of two others (D or E) must also be present in the dominant form in order to have complete pollen fertility. Thus, two of the three modifier genes would be duplicates. Similar mental exercises could explain some of the other ratios obtained but are of little value here. The point in presenting this tentative explanation is to demonstrate that one can fit genetic ratios to segregations that have been obtained. The next step should be to test these hypotheses by means of the usual methods of progeny testing individual F_2 and backcross plants. It may well be that in those cases in which several modifiers are involved, an exact annotation of all of the genetic factors will not be possible, due to the numerous small differences in tassel fertility which would result and which would be hard to distinguish as separate classes. In addition, such small genetic variations might be masked by environmentally induced variability more easily than larger differences would be. The fact that small numbers of plants in NR^T × FR

hybrids are not completely fertile could also upset genetic studies, if this should mean that a few genetically VC plants sometimes will appear to be IVC. The reason for the presence of small numbers of partially fertile plants in the F_1 hybrids is not yet clear, however. Their appearance conceivably could be due to heterozygosity of one or the other of the P_1 lines, but the lack of a sharp break between class IV and class V tassel types makes it seem more likely that the F_1 hybrids merely are exhibiting a normal range of variation for a uniform genotype when exposed to a variable environment. Once again, it is apparent that individuals of each type must be progeny tested. In the case of $C106^T \times$ Ky21, (which had an unusually large proportion of partially fertile plants) it may be that the Ky21 plant used to make this cross did not have a full complement of dominant FR modifiers, at least with respect to the C106 plant with which it was crossed. WF9 appeared to supply all modifiers needed, for in the 3-way cross, ($C106^T \times$ Ky21)WF9, (tracing back to the same Ky21 plant) nearly all pollen shedding plants were fully fertile.

There is another and a very important pitfall in the study of the genetics of fertility restoration for those cases in which segregating generations produce large numbers of partially fertile tassels. This is the fact that some genotypes tend to have appreciable numbers of abnormal anthers, even in normal cytoplasm. This is especially true for some inbred lines, with certain unfavorable seasons bringing out the trait quite strongly. The abnormal anthers (and the pollen in them) are similar in appearance to anthers which are partially fertile or sterile due to the action of Cyt^T. Therefore, all segregating populations should be grown in both Cyt^T and Cyt^N in order to be sure one is not confusing two separate types of inheritance. In general, however, hybrid material, and most BC_1 and F_2 populations, do not have abnormal anthers (in Cyt^N) even though their inbred parents may have exhibited them. Among the Cyt^N lines used in the crosses reported herein, WF9 and Ky21 tended to have several sterile or weak exserted anthers, especially during unfavorably hot and dry seasons.

And, finally, all backcross tests should be made in at least two ways, so that one can compare segregation and gamete transmission both through the pollen and through the egg in Cyt^T; i.e., both $C106^T$ ($C106^T \times$ Ky21) and ($C106^T \times$ Ky21)C106 should be made. Although it has been shown that the WF9 "reciprocal" backcrosses did not appear to differ, each of the other backcrosses must be tested in the same way. It also would be well to make the type of cross: $C106^T$ (C106 $\times$ Ky21) in order to test for segregation in Cyt^N as contrasted to segregation in Cyt^T.

SUMMARY

Five inbred lines (in so far as they were sampled) were shown to have the same alleles of the dominant genes required for restoration of pollen fertility to the inbred $WF9^T$ (cytoplasmically pollen sterile WF9). The five fertility restorer (FR) lines are Ky21, K55 (Agricultural Experiment Station lines), BH2, F5DD1 and WG3 (Pioneer Hi-Bred Corn Company lines). Backcross tests of the FR lines using five different sterilizable inbreds as recurrent parents have demonstrated that the fact that two or more inbreds are equally sterile in T cytoplasm does not mean that they necessarily are isogenic for all FR genes. Therefore, any statement of the number

and location of the FR genes in a given FR line is applicable only to the specific sterilizable inbred line used as tester and further, only to the specific plants which were tested, until such time as cross comparisons between plants and between lines have been made. Additionally, the environment in which segregating populations are grown can influence the number of FR genes that are revealed. Preliminary breeding tests indicate that, at the least, two dominant complementary genes plus one or (probably) more dominant genes which modify the action of one of the dominant complementary genes are required for full pollen fertility in T cytoplasm when plants are grown in Iowa during rather hot dry summers.

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KYNURENINE FORMAMIDASE IN MUTANTS OF DROSOPHILA[1]

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Received January 30, 1956

MANY workers have shown that the brown eye pigment of *Drosophila melanogaster* is a derivative of tryptophan, and that gene mutations affecting the conversion of tryptophan to kynurenine (the v^+ substance) in Drosophila manifest themselves as deficiencies in this pigment (cf. EPHRUSSI 1942; GREEN 1949; KIKKAWA 1950a). Recent examination of the enzymes responsible for this conversion in mammalian liver has indicated that these reactions involve a peroxidation of tryptophan to N′-formylkynurenine (KNOX and MEHLER 1950), and the hydrolysis of the latter compound to kynurenine and formic acid (MEHLER and KNOX 1950). The enzyme for this hydrolysis, kynurenine formamidase (JAKOBY 1954), is apparently specific for formylated aromatic amines and is able to hydrolyze formylanthranilic acid, formylorthanilic acid, formylnitroaniline, and others, although at a much slower rate than it acts upon formylkynurenine. This enzyme is quite active in cell-free extracts of Drosophila, and some of its properties will be described below. Unfortunately it has not been possible to demonstrate the peroxidation of tryptophan to formylkynurenine in cell-free extracts (assayed by tryptophan disappearance) even though larvae, pupae, and adults have been examined in various ways.

Kynurenine formamidase was assayed in various strains in which the production of kynurenine is known to be altered. These included the vermilion mutants, in which the formation of kynurenine is blocked completely, and ruby, claret, and others, in which the amount is merely lessened (BEADLE and EPHRUSSI 1937). In order to determine the differences between strains, a variety of other strains in which kynurenine production is normal was also examined. The study was extended to include stocks of *D. virilis* because of a report by KIKKAWA and his associates (1950b) stating that the mutant cardinal of that species "seems to lack the enzyme which converts formylkynurenine into kynurenine". However, no details were presented by those authors.

The experiments of the present investigation demonstrated that kynurenine formamidase is present in all of the strains examined. This indicates that the reaction block in the pseudoallelic vermilion mutants, as well as the other strains, is in the conversion of tryptophan to N′-formylkynurenine.

[1] Supported in part by a contract No. At(30-1)-1472 from the Atomic Energy Commission.

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MATERIALS AND METHOD

Sources of chemicals

The N′-formyl-DL-kynurenine used in these investigations was the gift of DR. W. E. KNOX. The presence of kynurenine in the preparation was taken into account. Some of the formylanthranilic acid used was donated by DR. W. J. JAKOBY. An additional supply of this compound was prepared according to a method modified from VON MEYER and BELLMAN (1923). All other chemicals were of commercial origin.

Collection of flies

The wild type strain of *D. melanogaster* used in these investigations was an Oregon-R stock maintained in this laboratory. Most of the *D. melanogaster* strains used were obtained from the California Institute of Technology; the *D. virilis* strains, from the University of Texas.

Flies were raised at room temperature (except where noted otherwise) on a standard medium of cornmeal, dextrose, and agar, supplemented with 3.0% brewers' yeast. Collections of adults were usually made every day and then stored at −15°C until used. Animals frozen for over one year showed an enzymatic activity comparable to that of freshly frozen ones.

Enzyme preparation

The enzyme was prepared by grinding the frozen adult flies in a chilled mortar, and then homogenizing in a glass tissue-grinder with 10 times their weight of 0.1 *M* phosphate buffer, pH 7.3 to 7.6. The homogenate was centrifuged at 20,000 × g for 20 to 30 minutes, after which the cellular debris and the thick lipid layer were discarded. The supernatant solution, which contained about 5 mg of protein per ml, was used when the specific activity of the enzyme in the fly was determined. When the properties of the enzyme were to be examined, the homogenate (darkly colored with eye pigments) was further treated by dialyzing for two to three days against four or five changes of large volumes of distilled water. The eye pigments were removed, and about 80% of the proteins were precipitated, by this treatment; the kynurenine formamidase remained in solution with little loss in activity.

Enzyme assay

The assay of kynurenine formamidase is based on the absorption of kynurenine at 360 mμ. When ascertaining the properties of the enzyme the dialyzed preparation was assayed in a manner similar to that described by MEHLER and KNOX (1950). To a 1.0 ml cuvette were added 0.1–0.2 ml of the enzyme, 1.4 μ moles of N′-formylkynurenine and enough 0.1 *M* potassium phosphate buffer to bring the volume to 1.0 ml. A change in optical density of 0.440 at this wavelength indicates the formation of 0.1 μ moles of kynurenine per ml. A similar procedure was used for following the hydrolysis of formylanthranilic acid, except that 10 μ moles of this compound

were used and readings were taken at 330 mμ. A change in optical density of 0.150 indicates the formation of 0.1 μ moles of anthranilic acid per ml.

When stocks were examined for specific activity, however, a different procedure was employed. It was thought unwise to use dialyzed homogenates because of the possible presence of a diffusible inhibitor of the formamidase. Since the eye pigments present in the crude homogenate absorb in the ultraviolet, and because complete removal of particles by centrifugation was sometimes difficult, it was found to be more convenient to assay the crude homogenate in a test tube containing five times the amounts of materials used in the cuvette described above. The enzyme was added last, after the temperature had equilibrated to 30°C. Aliquots of 1.0 ml were taken 15 seconds after mixing and at 10 or 15 minute intervals thereafter, for 30 to 45 minutes. The reaction was stopped by deproteinizing these aliquots with 1.0 ml of 0.18 *N* sodium hydroxide and 1.0 ml of 5% zinc acetate (MEHLER and KNOX 1950). The amount of kynurenine produced was determined from the optical density of the resulting neutral protein-free solution at 360 mμ. The unit of enzyme activity, when assayed under these conditions, is defined as that amount of enzyme which will produce a change in optical density of 0.001 in five minutes. Specific activity is the number of enzyme units per mg of protein. Protein was determined by the method of LOWRY *et al.* (1951), using blood serum protein as a standard.

RESULTS

General properties of the enzyme

The general properties of this enzyme preparation were examined only sufficiently to ascertain that the reaction studied was the hydrolysis of N′-formylkynurenine. In all of these experiments enzymatic assays were carried out in the spectrophotometer cuvette.

The enzyme exhibits little change in activity when the pH varies from 7.0 to 7.6; there is a slight maximum at 7.3. Broad pH optima, such as this, have previously been reported for rat liver formamidase (pH 6 to 8, MEHLER and KNOX 1950) and Neurospora formamidase (pH 7.3 to 7.8, JAKOBY 1954).

The Km calculated by the method of LINEWEAVER and BURK (1934) is 3.1×10^{-4} *M*; the value reported for the Neurospora enzyme is 1.1×10^{-4} *M* (JAKOBY 1954).

The enzyme extracted from Drosophila appears to be more specific than the analogous enzymes obtained from rat liver or Neurospora, in that the Drosophila formamidase does not detectably hydrolyze formylanthranilic acid under the conditions described (fig. 2). However, formylanthranilic acid inhibits the hydrolysis of formylkynurenine by 60 percent at 10^{-2} *M* and by 15 percent at 10^{-3} *M*. Other reported substrates for formamidase have not been tried.

The enzyme is inhibited completely by 10^{-2} *M* sodium bisulfite, but not at all by 10^{-2} *M* sodium formate or 10^{-2} *M* thiourea. This last finding proved very useful when assaying the formamidase in larvae, since addition of thiourea inhibited the

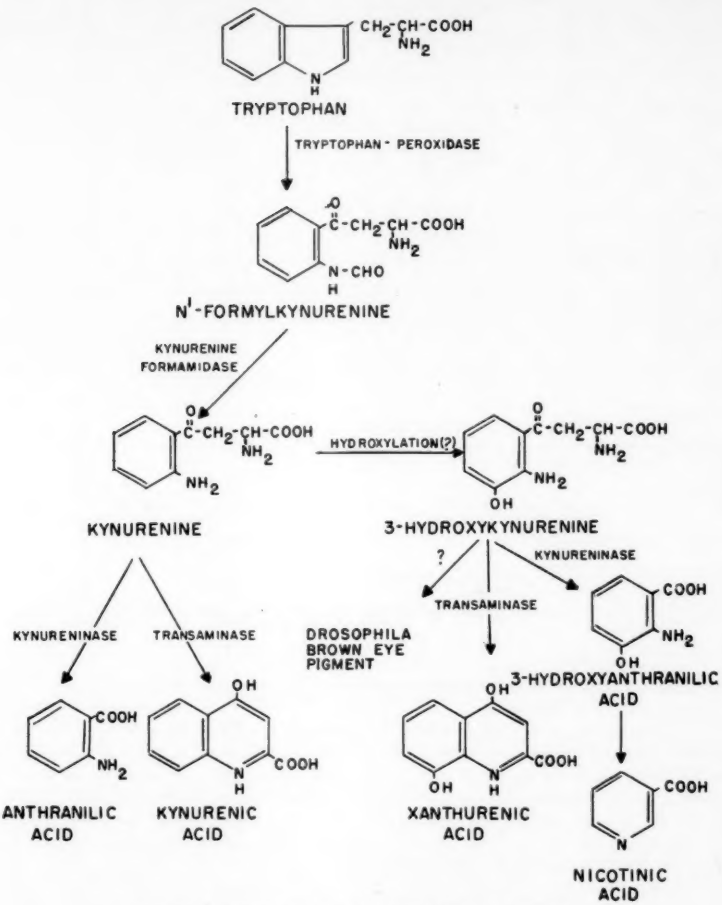

FIGURE 1.—Some aspects of the metabolism of tryptophan.

formation of substances (probably quinones), produced through the action of tyrosinase, which condense with kynurenine (GLASSMAN 1955).

The effect of age and temperature on the specific activity of kynurenine formamidase

In order to ascertain what effects the temperature at which the flies were raised, and the age of the fly after emergence, might have on the specific activity of the formamidase, flies were raised at 17–18°C, 22–23°C, 24–26°C, and 29–30°C, and collected at frequent intervals. Some flies were frozen immediately, while others were aged for 10 days at the temperature at which they were grown. Assays were per-

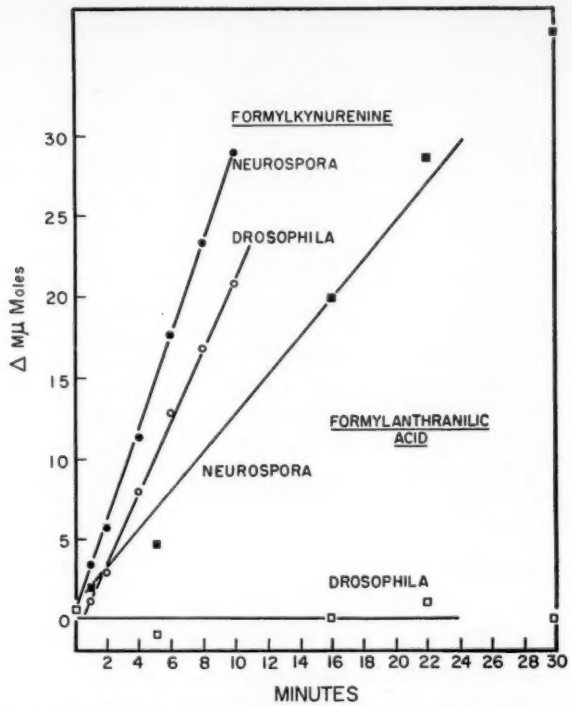

FIGURE 2.—Activities of kynurenine formamidase from Drosophila and Neurospora on N′-formylkynurenine and formylanthranilic acid. The Drosophila extract was prepared as described in the text. The Neurospora extract was prepared by homogenizing wild type mycelium with four times its weight of 0.1 *M* phosphate buffer, pH 7. The solution was centrifuged at 20,000 × g for 20 minutes and the supernatant was stored at −15°C until used. Assays were conducted in 1.0 ml volumes containing 0.2 ml of extract, 1.4 μ moles of N′-formylkynurenine or 10 μ moles of formylanthranilic acid, and 0.1 *M* buffer, pH 7.6. The data are presented as the change in millimicromoles of substrate against time; the two upper curves and the two lower curves are for the hydrolyses of N′-formylkynurenine and formylanthranilic acid, respectively.

TABLE 1

The effect of age of the adult flies and the temperature at which they were raised on the specific activity (number of enzyme units per mg of protein) of kynurenine formamidase

Age (days)	Specific activity			
	17–18°C	22–23°C	24–26°C.	29–30°C
0–1	24	22	23 26	24
	26	23	23 27	24
			24 27	24
			24 28	
			25	
9–10	25	25	28	23
	27	26		24
				27

formed as described above. The data are presented in table 1, where it can be seen that the specific activity of the enzyme is not detectably affected either by the temperature at which the flies are grown or by the age of the fly after emergence (up to 10 days). The specific activity of the wild type under the conditions stated is between 22 and 28 enzyme units per mg of protein.

Assay of mutant strains

The data for the specific activity of kynurenine formamidase in a number of mutant stocks of *D. melanogaster* and *D. virilis* are presented in figure 3. All stocks (within species) had similar levels of enzyme activity. Some differences exist, but the wide variation within strains makes it difficult to conclude that any stock differs significantly from the wild type. On the other hand, there does appear to be a significant difference between the two species. Whether the lower specific activity found in *D. virilis* is related to the larger size of the individuals or to some other factor is not known.

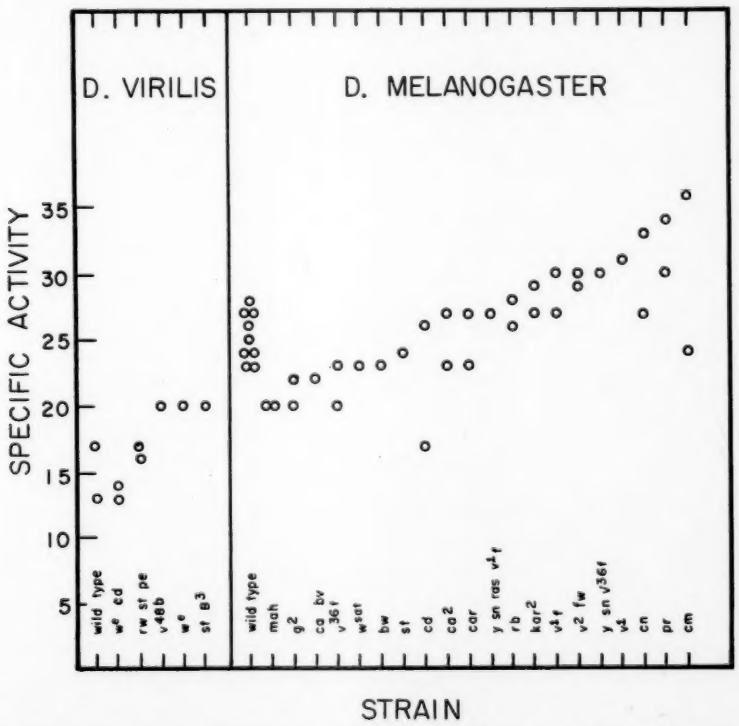

FIGURE 3.—The specific activity (number of enzyme units per mg of protein) of kynurenine formamidase in various strains of Drosophila.

DISCUSSION

In spite of the vast literature which implicates kynurenine and 3-hydroxykynurenine as precursors of the brown eye pigment in Drosophila, only few data are available which indicate that tryptophan is a precursor of these compounds in this organism. Not until relatively recently was it demonstrated that vermilion flies, which cannot form kynurenine, accumulate high levels of a compound which reacts positively in the colorimetric test of HORN and JONES for tryptophan, and which is presumably that compound (GREEN 1949). That this compound is a precursor is borne out by the fact that when the production of brown eye pigment is increased by a suppressor gene (*su*^{2}-*v*), the amount of this compound accumulated by vermilion flies is decreased. Further evidence that tryptophan is converted to kynurenine in Drosophila is provided by KIKKAWA (1950), who, by feeding tryptophan to cinnabar larvae, was able to demonstrate an increase in the amounts of kynurenine normally accumulated by this strain.

The demonstration that in Drosophila there is an enzyme capable of hydrolyzing N′-formylkynurenine to kynurenine and formic acid confirms these interpretations. However, tryptophan-peroxidase has not yet been demonstrated in cell-free extracts even though different methods of extraction of various stages have been tried. Similar attempts by M. M. GREEN, E. KNOX, and E. CASPARI (personal communication), working independently, have also proved fruitless. Whether this is because the concentration of the enzyme is too low to be detected, or because the enzyme is unstable, or because of some other factor which has not been recognized, is not known.

Kynurenine formamidase was present in all of the mutant stocks examined. Most of the strains were assumed to contain the enzyme because they produce sufficient kynurenine; they were used only to determine the variation to be expected from strain to strain. Vermilion strains, however, lack the ability to make kynurenine, while other strains, such as carmine, claret, and ruby, do not make quite as much as does the wild type (BEADLE and EPHRUSSI 1937). These latter stocks were examined in the hope that an absence of formamidase might turn out to be responsible for this condition. Since the enzyme was found to be present in all stocks, it may be concluded that the mutant genes block the reaction prior to the formation of formylkynurenine, i.e., in the tryptophan-peroxidase system. These data confirm the observations of GREEN (1952) who reported that the various vermilion pseudoallelic mutants could make brown eye pigment from formylkynurenine supplied in the medium. His data remained questionable, however, since in the acid medium used, formylkynurenine spontaneously hydrolyzes to formic acid and kynurenine, which is also active.

Formylanthranilic acid, which is hydrolyzed by kynurenine formamidase from rat liver or Neurospora, does not appear to be a substrate of the Drosophila formamidase; however, its presence does inhibit the hydrolysis of formylkynurenine. A similar situation has been recorded for kynureninase, which when extracted from Neurospora can convert formylkynurenine to formylanthranilic acid (JAKOBY and BONNER 1953), but when obtained from Pseudomonas is unable to do this (HAYAISHI

and STANIER 1952). A more detailed analysis of this point, supplemented with data on other reported substrates of the enzyme, would be of interest.

SUMMARY

1) Cell-free extracts of *Drosophila melanogaster* were examined for the enzymes that convert tryptophan to kynurenine. Only kynurenine formamidase was found. The tryptophan-peroxidase system could not be demonstrated in these extracts.

2) The Drosophila kynurenine formamidase has a broad pH optimum between 7.0 and 7.6. The Km was 3.1×10^{-4} *M*. The enzyme is inhibited completely by 10^{-2} *M* sodium bisulfite, but not by 10^{-2} *M* sodium formate or thiourea. It does not utilize formylanthranilic acid as a substrate, although it is inhibited by this compound. On the other hand, when extracted from rat liver or Neurospora, kynurenine formamidase does hydrolyze formylanthranilic acid.

3) The specific activity of the enzyme does not vary with the age of the adult, nor is it changed by raising the flies at various temperatures between 17°C and 30°C.

4) The specific activity of the enzyme was assayed in various mutant strains. In no strain was the activity significantly different from that of the wild type. Strains of *D. virilis* had lower specific activities than those of *D. melanogaster*.

ACKNOWLEDGMENTS

The author would like to express his gratitude to DR. BENTLEY GLASS, whose interest and counsel was invaluable throughout this investigation; and to DRS. W. D. MCELROY and H. K. MITCHELL for critically reading the manuscript. He is also indebted to ANN JUDITH TERRELL for her assistance, and to REBECCA K. RITTERHOFF and PAULINE LEE for the Drosophila stocks and food.

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EFFECT OF SEED TREATMENTS WITH X-RAY AND PHOSPHORUS 32 ON TOMATO PLANTS OF FIRST, SECOND, AND THIRD GENERATIONS[1]

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Received January 30, 1956

MANY observers have reported an increase in variability following exposure of pollen or seeds to the effect of ionizing radiation. In several respects the tomato is favorable material for such studies. It is self-pollinating, and recessive mutants can therefore be uncovered promptly. It is a perennial and can be propagated by cuttings, so that plants of a previous generation can be retained, and two generations can be grown in one year. The normal cytological and genetical behavior is well known. In a recent report (Tomato Genetics Cooperative Report No. 4, 1954) 108 mutant genes of the tomato are listed, and the number is increasing rapidly. LINDSTROM (1933), with radium, and MACARTHUR (1934), with X-rays, succeeded in inducing mutations in this species.

Exposure of pollen to radiation has been much more used than exposure of seeds. The latter method seemed to offer certain advantages in using a radioactive isotope, since, when seeds are soaked in a solution containing P^{32}, the radioactive isotope may enter the nuclei of the embryo before and during germination and the multicellular organism is exposed to decreasing amounts of beta radiation during a period of several weeks.

The studies reported in this paper include a single experiment with X-rays and nine with phosphorus 32 during three generations of mature plants.

EXPERIMENT WITH X-RAYS

Seeds of an inbred line of First Early, a highly fruitful tomato variety, were exposed to X-radiation. Eighty-one seeds were kept on moist filter paper for two days at 80°F before irradiation, and the same number were irradiated in a dry state. Each lot received somewhat less than 10,000r at the rate of 300r per minute. The source of X-rays was a Coolidge tube with tungsten target, operating at 80 KV and 3 milliamperes and without a filter. The results of this experiment were as follows.

After planting, 83 percent of the dry-treated seed and 77 percent of the wet-treated seed emerged. Only one of the 45 seeds planted as a control failed to grow. Germination of the treated seeds, especially of the wet lot, was slower and more irregular than that of the control seeds.

The plants that grew from the control seeds were normal in every way, and most of those from dry-treated seeds appeared to be normal. Most of the 46 plants from wet-treated seeds developed very slowly and many of them were abnormal in meiosis and partially male sterile; 5 were almost totally unfruitful, 5 were only fairly fruitful, and 36 had a full crop by the end of the season.

[1] Paper No. 898, University of California Citrus Experiment Station, Riverside, California.

All of the plants which grew from wet-treated seeds were diploid, having 24 chromosomes. Four plants had very few pollen mother cells and these remained in masses during meiosis. In nine plants non-reduction was frequent, and in three of them, there was one unequal pair. Five plants had one tetravalent, one had two trivalents or a hexavalent, one had an inversion bridge and fragment, and two had a trabant-like fragment which was sometimes free. In a few cells in three plants the chromosomes were elongated and formed stickiness bridges at first anaphase.

The seeds that were irradiated and the plants that developed from them were called the R_1 generation; the generation from selfing R_1 was called R_2, and so on. Twenty R_1 plants of the "wet" series produced seeds from open pollination. Small R_2 populations of from 9 to 18 seeds were planted from each, making a total of 235 seeds, of which 206 germinated.

One R_2 family contained 3 plants closely resembling the gene mutant wiry (*w*), and 30 normal plants. The wiry-like mutant differed from wiry (*w*) in that the ovary was less apocarpous, and, like the thread-leafed mutant described by SCHIEMANN (1933), was partially female fertile. A normal plant of this family was selfed and gave in R_3 26 wiry-like and 59 normal plants, or approximately the expected monohybrid ratio.

No other previously known mutants occurred in the progenies of the R_1 plants. A few apparent mutants occurred in 10 of the 20 R_2 families, these mutants including one wholly sterile plant, one having a variegated leaf, several plants having fruit that was somewhat smaller than normal, and some on which the fruit ripened later than that of the control plants.

EXPERIMENTS WITH P^{32}

Dosage, seed germination, and subsequent growth

Seeds of tomato varieties originating from single plants of lines inbred for three to six generations were soaked in solutions containing P^{32}. (This isotope was supplied by the Oak Ridge National Laboratory, Oak Ridge, Tennessee, as phosphate in acid solution, denoted "P-32-P-1 processed, high specific activity"; half life 14.3 days.) In the nine treatments, the initial dose per seed, expressed as microcuries of P^{32} in solution per number of seeds soaked, ranged from 2.7 to 10.8 microcuries. The temperature during soaking was 70–85°F. Enough distilled water was subsequently added so that some liquid remained in the container at the end of treatment. The non-treated seeds received distilled water only.

The percentage of seed germination was reduced by an initial dose of 5.1 microcuries per seed and 9 days' soaking, but was normal with an initial dose of as much as 10.8 microcuries per seed and 5 days' soaking (table 1, T3, T5, T7, and control 53.61.) It is probable that the longer soaking time interfered with respiration and growth. Germination of treated seeds, even with the lowest dose, was slower and more irregular than in the control. Subsequent growth was much slower, especially with the higher dosage. The mortality both before and after transplanting to the field was also higher than in the control.

The initial dosage per seed used in the nine experiments with P^{32} is shown in table 1. Reduction of fruitfulness was in general proportionate to dosage. Treat-

TABLE 1

Effect of P^{32} treatment of tomato seeds on germination and plant survival

Treatment	Family	Variety	Date of treatment	Number of seeds treated	Initial dosage μC		Total volume of liquid, ml	Duration of treatment, days	Seed germination percent	Number of plants	
					Total	Per seed				In greenhouse	In field
T1	52 C	Riverside	3–1–52	50	271	5.4	2.0	3	94	—	36
T2	52 B	"	3–1–52	50	136	2.7	2.0	3	100	—	34
Control	52 A	"	3–1–52	50	0	0	2.0	3	96	—	12
T3	52.129	"	5–13–52	50	256	5.1	2.0	9	58	29	21
T4	52.130	"	5–13–52	50	256	5.1	2.0†	9	42	21	21
Control	52.128	"	5–13–52	—	0	0	—	6	100	—	9
T5	53.62	Canary Export	5–4–53	50	540	10.8	1.0	5	98	45	33
T6	53.63	"	5–9–53	25	‡	—	—	9	88	21	21
Control	53.61	"	5–4–53	50	0	0	1.0	5	92	—	23
T7	54.20	"	3–10–54	70	756	10.8	1.4	5	100	20§	0
Control	54.21	"	3–10–54	70	0	0	1.4	5	95	4	—
T8	54.220	"	9–15–54	50	540	10.8	1.0	3	98	49	—
T9	54.221	"	9–15–54	50	234	4.7	1.0	3	98	49	—
Control	54.219	"	9–15–54	25	0	0	.5	3	96	4	—

† Includes 1 cc nutrient solution complete but without phosphorus.

‡ Residual liquid after soaking 50 seeds in 540 microcuries for 5 days.

§ Fifty treated seeds were sacrificed for assay of radioactivity in seed.

ments 1 and 2 were given at the same time and with portions of the same sample of active material, but in T1 the initial dose of P^{32} was twice that in T2. In T2, 44 percent of the R_1 plants were as fruitful as the control and the rest were less fruitful. In T1 only 9 percent were as fruitful as the control and the rest were less fruitful. On the other hand, two lots of seed which received the same initial dose per seed (T5 and T7) but with different preparations and possible dilutions of P^{32}, gave very different results. In T7, germination was perfect, but every plant died in the cotyledon stage. The control plants developed normally. In T5, 76 percent of the plants developed beyond the cotyledon stage, and all but six of these survived transplanting to the field. The sample of P^{32} used in T7 gave a heavy precipitate at pH 7, whereas the other sample gave only a light precipitate. In T8 with the same initial dose and only 3 days' soaking, 98 percent of the plants developed well.

In T7, 70 seeds received an initial dose of 756 μC or 10.8 μC per seed and the soaking period was 5 days. The average total activity during the 5 day period, therefore, was 675 μC and the activity per seed was 9.6 μC. If 1 μC of P^{32} produces 43 roentgens per day, during the 5 day period of treatment each seed received $43 \times 9.6 \times 5 = 2060$r. After the 5 day treatment in T7 25 of the 70 seeds were removed from the liquid without draining, reduced to ash and counted with a Nuclear Geiger Counter. These seeds were found to contain 27.83 μC. Another lot of 25 seeds was thoroughly washed in distilled water, drained, ashed, and counted. The amount of activity in these was 10.14 μC, so that washing and draining removed 64 percent of the P^{32}. The amount present in the washed seeds leads to the conclusion

that of the 595 μC remaining after soaking 70 seeds for 5 days, 28.4 μC or about 4.8 percent was actually in the seeds. Comparison of the dosage in r in the X-ray experiment previously described with T5 or T7 shows that the P^{32} treated seeds received during the 5 days' soaking period about twice that of the X-rayed seeds.

The differences in growth of treated seeds in the same culture is believed to be due to differences in the amount of P^{32} absorbed. Individual seeds after soaking gave different readings with a portable beta radiation monitor. Previous work by GUSTAFSSON *et al.* (1950) showed that barley kernels soaked in a similar manner varied widely in their intake of P^{32}.

Somatic changes in R_1 and R_2

The nontreated and treated seeds, some of which had germinated, were planted about 2 inches apart in flats in the greenhouse. In some R_1 plants the cotyledons were narrower than usual, slightly distorted, and abnormally pale green. Most of these chlorotic seedlings died in the cotyledon stage and all were sterile. In T5, six weeks after planting, one third of the young plants had twin or triple shoots. Apparently some cells of the original apical meristem were injured by the treatment.

Somatic changes were frequent in which only a portion of the plant was involved. The extent of the change varied from a single leaflet to an entire branch on an

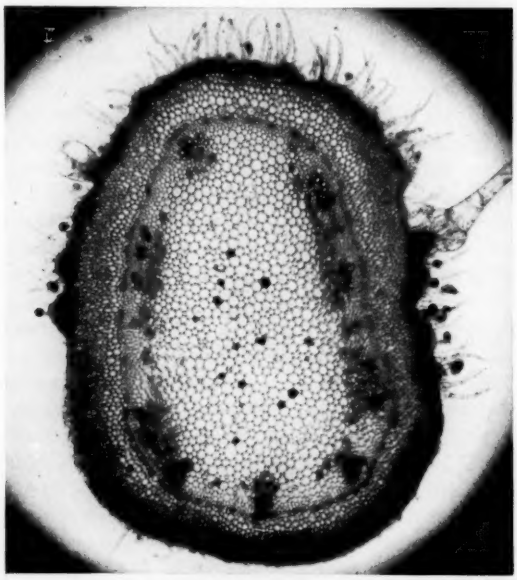

FIGURE 1.—Transverse section of fresh stem of chimera R_2 plant 27G1. Top, normal pubescent sector with chlorophyll in cortex; bottom nearly glabrous green; right upper side, above large trichome, pubescent albino; left lower side glabrous albino sector.

otherwise normal plant. Chlorophyll variegation was by far the most frequent and consisted of normal and rather dull pale green tissue. In T5, seven of the 34 plants which passed the cotyledon stage were variegated and this condition persisted in plants grown from cuttings of five of them. In T8, 27 of the 49 young seedlings were variegated and in all but five of them the variegation was persistent.

Another somatic change in R_1 plants was an almost complete loss of pubescence on part of the plant. In one case both the number of loculi in the ovary and the shape of the leaves was changed on different branches of the same plant.

Somatic changes occurred much less frequently in R_2 than in R_1. Among 750 R_2 plants, only 12 were variegated. Seven of these came from 38 not variegated R_1 parents and four from 17 variegated R_1 parents. Wholly pale green or non-pubescent branches were sterile.

The most remarkable somatic change was found in R_2 plant 54.27.G1, from an apparently normal R_1 plant. The leaves were very irregularly variegated with green and white, often distorted and nearly white at the edges. Some of the immature fruits were variegated. The stem and leaves contained green and non-green cortical and mesophyll cells. In addition, well-defined almost non-pubescent areas occurred on the stem and leaves (fig. 1), and occasionally this plant produced a weak and sterile non-pubescent pure green shoot with smaller leaves. No normal leaves occurred. This plant was nearly pollen sterile, but produced seeds from pollination with a control plant. The progeny were non-variegated, but the only plant from selfing was variegated. In the same R_2 nearly all 11 plants were greyish-green and 8 were partially or wholly male sterile. No somatic changes were found in the controls. R_2 families from three variegated parents contained green and albino seedlings in the ratios 4:2, 10:4, and 15:1. No albinos occurred in R_2 from non-variegated parents.

Mutations in R_2 of T5

Seeds of the R_2 generation from selfing 24 plants from P^{32}-treated seeds, and seeds from variant branches of two of these parent plants were planted, making a total of 55 sowings and 750 R_2 plants. No seeds germinated from either the normal or variant branches of one R_1 plant, and none from another parent. In one family there were five plants resembling in flesh color of fruit the well known *r* (yellow flesh) mutant. One of these crossed with r^+/r gave one yellow-fleshed and one red-fleshed plant. Probably the new mutant is identical with *r*. A single plant having tangerine flesh occurred in another R_2. It was identical in flesh and flower color with the tangerine mutant (*t*) in the variety Golden Jubilee and was proved by an allelic test to be at the same locus, but it was virescent. An allelic test and chromatographic separation of the ripe fruit pigments in an adsorbent column indicated identity with a virescent tangerine mutant obtained from DR. F. O. HOLMES, Rockefeller Institute, New York. As only 54 different R_1 plants were tested and one of them was probably r^+r and another t^+t, a high mutation rate in the embryonic cells of the treated seeds is suggested. A mutant resembling *a* (anthocyanless) appeared in R_2 from T7, but an allelic test showed that it is not identical with *a*.

Besides the two which probably are identical with known mutants, several others

occurred which are believed to be gene mutants. Meiosis was normal in all of them. The most striking mutant had bright-yellow foliage and a pale-yellow corolla and stigma. The hypocotyl contained less anthocyan than normal a^+ sibs. It may appropriately be called "aurea" (*au*). Only the oldest leaves turn green, either in the field or in the greenhouse. From selfing aurea, 8 aurea seedlings were obtained; the backcrosses ($au^+ \times au$) $\times$ *au* and ($au \times au^+$) $\times$ *au* gave, respectively, 5:9 and 6:6 *au*:au^+. Progenies from reciprocal crosses with *l* (lutescent) or with the virescent tangerine were all green or wild type. Apparently aurea is a new mutant and viable especially in outcrosses despite its deficiency of green color. It seems to be a good seedling character. Aurea occurred in the same R_2 of only 15 plants as the *r* mutant.

Brachytic mutants occurred in five different R_2 families from T5. Two brachytics occurred in one R_2, and one of them had more loculi in the ovary than the control. Two brachytic mutants from different R_2 families were recessive to the control type, but some brachytics did not breed true. One brachytic mutant occurred among the check plants. A multilocular variant in another R_2 proved to be a trisomic, rather like triplo-5 (RICK and BARTON 1954).

Other mutant types in R_2 differed from the control line in the number of flowers in the inflorescence, leaf shape, male sterility, time of maturity of the fruit, fruit size, and in having blue-green leaves. Some of them, especially those having earlier maturity and male sterility, may have useful practical applications. No mutants were found in the control plants.

Pollen sterility

Pollen of fully opened flowers was examined in iodine solution (1 gram KI, 0.3 gram I, and 100 ml H_2O). Such preparations must be examined immediately. Grains that were of normal size, round, and light yellow were counted as normal. Immature grains near maturity and defective grains which have failed to mature contain starch grains which stain purple; younger grains tend to collapse in iodine. When counts of normal and empty pollen were needed acetocarmine was used. Both methods probably tend to overestimate the proportion of viable grains.

A nominal scale was used for rating pollen quality. This scale was based on the percentage of normal pollen grains found in three or four flowers taken from different

FIGURE 2.—53.62.24 A. Diakinesis, dissimilar homologues unpaired. B. and C. First anaphase C is far more common than B.

parts of the young plant, as follows: 75–100 percent (++), 50 percent (+), 25 percent (±), and 0 percent (0). Pollen was rated before the amount of fruit set was known. A similar scale was used for rating fruitfulness, beginning with the highest degree (++), followed by three intermediate degrees (+, ±, and −), and concluding with no fruit (0). It is clear that in many plants pollen sterility was a major cause of reduction in fruitfulness and fertility, and that they are closely related. Plants having very few fruits usually produced no seeds.

In most cases the pollen was the same for the entire plant, but an occasional R_1 plant had two distinct types of pollen. In a few plants two types of chromosome structure and behavior could be shown to account for the difference in pollen fertility. The hairy or more normal-appearing part of one plant, for instance, had an unequal pair of chromosomes and no pollen or fruit (fig. 2) whereas the glabrous portion had ± pollen and normal meiosis and bore two seedless fruits.

In the earlier experiments with P^{32} the variety used set so little fruit that comparisons of pollen and fruit set were difficult. Nevertheless, the records suggest clearly that in these families, poor pollen and unfruitfulness were associated. Also, T2 with 2.7 microcuries per seed caused less damage to pollen fertility and fruitfulness than T1 with 5.4 microcuries. The correspondence between grade of pollen and grade of fruitfulness in individual plants was very good.

In a second series involving the same variety (table 1, T3 and T4), 17 of the 33 plants recorded from treated seeds had poor pollen and were low in fertility. Four of these plants were wholly male sterile, and the other 13 had less than 12 percent good pollen and extremely few seeds. The control plants were ++, both in pollen and in fruitfulness.

For the later experiments a very fruitful variety, Canary Export, was used. The plants were extraordinarily disease free in 1953 and 1954, and the controls were uniformly fruitful. In T5 the heavy initial dose of 10.8 microcuries per seed had a proportionately great effect on pollen fertility. The results are shown in table 2. T5 reduced pollen fertility and fruitfulness more drastically than T6. In T5, 10 plants were wholly male sterile, and even by late August only one plant in the whole population had a full crop. Three of the plants without fruit in T5 may have been female sterile. In T6 there was apparently enough activity in the residual liquid to affect

TABLE 2

Effect of P^{32} treatments on pollen fertility and fruitfulness of Canary Export variety of tomato

Treatment	Initial dosage μC per seed		Rating					Date recorded
			++	+	±	−	0	
Control	0	Pollen	8	0	0	0	0	7/14/53
		Fruitfulness	8	0	0	0	0	8/23/53
T5	10.8	Pollen	1	8	11	7	10	7/14/53
		Fruitfulness	1	9	3	8	13	8/24/53
T6	Residual from T5	Pollen	3	6	3	4	3	7/14/53
		Fruitfulness	8	7	0	1	4	8/24/53

TABLE 3

*Relation between fruitfulness of R_1 parents and pollen fertility and fruitfulness of R_2 progeny of Canary Export tomato in T5 — 10.8 μC per seed**

		R_1 fruitfulness ++ or +					Date of rating
		++	+	±	−	0	
R_2 plants	Pollen fertility	26	36	23	10	5	7/11/54
	Fruitfulness	27	43	6	8	14	7/12/54
	Fruitfulness	67	12	10	7	6	8/30/54
Control	Fruitfulness	5	3				7/12/54
	Fruitfulness	8	0				8/24/54
		R_1 fruitfulness ± or −					
R_2 plants	Pollen fertility	6	15	31	16	10	7/11/54
	Fruitfulness	18	29	6	12	15	7/12/54
	Fruitfulness	28	17	13	6	8	8/30/54

* For description of rating scale see text pages 580 and 581.

pollen fertility materially. Nontreated populations of Canary Export were highly fruitful.

In R2 after T5 none of the seventeen populations were entirely ++ in fruitfulness. Seven populations contained from 1 to 5 plants with no good pollen and no fruit. Six others contained 1 or 2 plants with poor pollen and extremely few fruits. One population from a ++ parent had one sterile mutant and two plants with greatly reduced fruitfulness.

The data in table 3 show that 66 percent of all plants from ++ or + parents, and only 39 percent from ± or − parents, had a full or ++ crop of fruit by August 30. A wide range of fertility grades was found in most families, and attainment of the ++ grade was much slower than in the controls.

Cytological irregularities in R_1 and R_2

It has not always been possible to find cytological abnormalities to account for unfruitfulness in either R_1 or R_2. Unfruitful plants with few buds, few p.m.c., green anthers in mature flowers, or abnormal flowers with or without pollen mother cells have been found in R_2. The meiotic process may appear to be normal although development of the plant is affected.

P.m.c. of the R_1 plants with poor pollen were examined in orcein. The data in table 4 show that translocations occurred more frequently than inversions and that plants with fragments were rare. All R_1 plants had 24 chromosomes.

Segregation of the members of an unequal pair of chromosomes in the parent gave rise in R_2 to a male sterile plant with 11 normal pairs and one exceptionally small pair (fig. 6) and another plant with an exceptionally large pair. A variegated plant in the same family had 11 normal pairs and a trivalent with two normal-sized and one smaller extra chromosome (fig. 5). Male sterility was apparently due to a homozygous deficiency. One male sterile R_1 plant was trisomic (fig. 8).

TABLE 4

The effect of irradiation on chromosomes in pollen mother cells (p.m.c.) and on pollen of tomato plants in R_1

Treatment	Initial dose μC per seed	Number of plants									
		P.m.c. few and in masses in prophase	Frequent non-reduction		Ring of four	Ring of six or two chains of three	Elongation and stickiness bridges	Inversion bridges with fragments	No good mature pollen	12 II and fragment	Two trabant like fragments
			equal pair	unequal pair							
X-ray (wet)	—	4	6	3	5	1	3	1	—	—	2
T3	5.1	—	4	2	2	1	—	2	1	—	—
T4	5.1	—	2	1	—	1	—	—	1	—	—
T5	10.8	5	6	2	6*	2	1	1	7	1	—

* One plant had two rings of four.

In another R_2 family from a parent with a slightly unequal pair of chromosomes, four abnormal plants occurred. Three of these were male sterile. Two of the male sterile plants had few normal buds and few p.m.c. and one plant was minute.

One R_2 plant, 54.27.G1 as already noted, was a mosaic of green, white, pubescent, and non-pubescent tissue (fig. 1). It was nearly male sterile but female fertile. Neither the parent plant nor the sibs in R_2 were variegated, and all sibs had 12 pairs of chromosomes. The mosaic R_2 plant has a small fragment which is often free, and two trivalents in meiosis (fig. 4A and 4B). Variegation may be due to abnormal distribution of the small fragment in the somatic cells. Bridges which occur in p.m.c. of the mosiac plant may be either simple or complex (fig. 4C), their form depending on whether the chromosome with the inversion was in a pair or a trivalent.

In another R_2 family, a variegated mutant had poorly paired chromosomes, frequent non-reduction, and the p.m.c. tended to remain in masses. Another plant in this

FIGURE 3.—54.23. A. Ring from parent of 54.23. (Only one ring was found.) B. Typical diakinesis in 54.23.2. C. Pachytene from 54.23.3 which had the same ring as plant 2. D.-G. Two types of rings and pachytene figures from plant 6. Note that pachytenes C and E and rings in A, B, and F are alike.

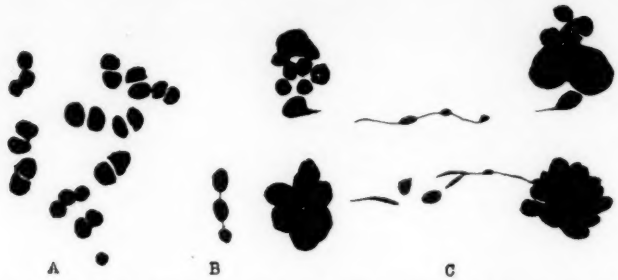

FIGURE 4.—27G1 A. Two trivalents and a fragment at diakinesis. B. The fragment in a trivalent. C. Bridges at first anaphase.

FIGURE 5.—54.36.6. Trivalent at diakinesis.

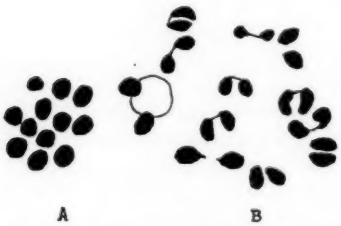

FIGURE 6.—54.35.3. The small pair is at the top of the drawing in both A and B.

FIGURE 7.—54.43.12. A. Trivalent at diakinesis. B. Pair and single chromosome at pachytene.

FIGURE 8.—52.130.3. A. Trivalent at first metaphase, involving two very large and one small dividing chromosome. B. Trivalent at pachytene.

family was trisomic, probably triplo-5 (fig. 7). This indicates that failure of normal pairing was also present in the parent.

In three plants out of 15 in another R_2 family, the four microspores of the tetrad failed to separate and no normal pollen developed. This condition is probably caused by a recessive gene.

R_2 from an unfruitful (±) plant with one ring of four chromosomes consisted of seven plants all of which had the same type of ring of four and unfruitfulness. Another R_2 family from a parent with one ring of four chromosomes consisted of three plants with one ring of four similar to the ring found in the parent (fig. 3A and C), one male sterile plant with two rings of four (fig. 3D–G), and two plants with normal pairing. One of these was very fruitful, the other male sterile. The latter was crossed with a normal. F_1, selfed, gave some plants with one and others with two rings of four.

DISCUSSION

An important difference between the X-ray and the P^{32} treatments was in duration. The seeds were exposed to X-ray for 33 minutes, whereas the P^{32} acted during the soaking period and until the absorbed fraction had completely disintegrated, a period of about six weeks. Nevertheless, these methods gave very similar types of chromosome changes. Table 4 shows that the proportion of each type of aberration which occurred in meiosis was also very similar after X-ray and P^{32} treatment. There was much male sterility after both types of treatment and well known mutants were induced.

X-ray treatment of seeds which had been wet for two days was much more effective than treatment of dry seed. This agrees with the results of SAX and BRUMFIELD (1943). It appears that hydrated cells are much more sensitive to irradiation than dormant cells.

Chlorophyll variegation was frequent in R_1 with the P^{32} treatment and usually involved a small part of the plant. Probably this is due to the large number of cell divisions and advanced stage of development of some cells during the relatively long exposure to P^{32}.

The most common immediate effect of P^{32} was variegation in green color. When whole branches were chlorotic, they were sterile. Possibly in plants which escaped with little or no apparent plastid injury, delayed cell division or development was a factor. EHRENBERG and NYBOM (1954) reported the prevalence in R_1 and R_2 of chlorophyll mutations in barley exposed to ionizing radiations. In tomatoes only a few variegated plants occurred in R_2.

With P^{32} the changes in chlorophyll and pubescence in R_1 and R_2 may be direct and permanent effects of radiation on the plastids and somatic cells. Similar somatic changes accompanied by sterility have occasionally been reported on nontreated plants. The albino seedlings in R_2 from variegated plants may simply have received injured plastids. Some recessive gene mutations that affect the chlorophyll, also a dominant *H* and a recessive *hl* (BUTLER 1952), that affect pubescence, do not cause sterility.

Many young seedlings in R_1 of T5 had two and a few had more than two phenotypically different parts. Due to death of the chlorotic part or overgrowh of more normal tissues, only 3 mature plants had two distinct phenotypes. In each case,

these two parts differed in pollen fertility. Male sterility was obviously due to segregation of a very unequal pair of chromosomes in one case. It seems probable that cells with large deficiencies or much fragmentation are eliminated at an early stage.

Triploids and male steriles are the most frequent abnormalities found in untreated cultures of tomatoes. Haploids, tetraploids, trisomics and deficients occur rarely.

After X-ray and P^{32} treatment, translocation was by far the commonest chromosomal change observed in meiosis of R_1 plants. Both GOTTSCHALK (1951) and BARTON (1954) report numerous translocations after X-ray treatment of tomato pollen mother cells or pollen. In our material, segmental interchange occurred between two, three, or four non-homologous chromosomes giving rise, respectively, to one ring of four, two chains of three or a chain of 6, or two rings of four in meiosis.

In R_2 the same types of chromosome aberration that were found in R_1 recur, and in addition trisomics, deficient trisomics, and homozygous deficients are found. One would expect to find occasional trisomics as long as weakness of pairing continues to exist in the line. Deficients would recur because plants with an unequal pair may be phenotypically normal. The latter have much nonviable pollen, but some of them can produce a fairly normal late crop of fruit.

In R_1 and R_2 after T5, the frequent plastid changes in some populations may have caused physiological disturbances which result in such conditions as (a) few p.m.c. that remain in masses, (b) greenish, browning, or separated anthers and (c) few flowers, and thus, might be responsible for some of the reduction in fruitfulness. Even some of the periodical nondisjunction might be due to lack of proper nourishment or to absence of some hormones during a critical time. However, this is certainly not always the cause, since in some plants in both R_1 and R_2 nondisjunction was clearly due to an obvious size difference in one pair. Probably many such differences were too small to be detected at the later stages of meiosis.

The R_2 data suggest an increased gene mutation rate caused by beta radiation of the seeds. In one R_1 plant from T5, gene mutations occurred, both to *r* (yellow flesh) in linkage group II and to aurea (*au*). The mutations *r* and *t*, rarely occur spontaneously. The virescence of the tangerine mutant suggests the possibility that two loci are affected. The tangerine *t* mutant, first listed by the W. Atlee Burpee Seed Company about 1940, is non-virescent, but another almost equally virescent tangerine type is known. The five yellow fleshed (*rr*) mutant plants were less fruitful than the ten red-fleshed sibs. The *r* mutation probably is much older than tangerine and has recurred in the Pearson variety, according to RICK (1947). He estimates (RICK 1945) a normal gametic mutation rate of 20 per 100,000 for genes affecting unfruitfulness. The wiry mutation which appeared in R_2 from X-ray treatment seems to occur more frequently, but the allelism of the various wiry mutants is not known. Another probable gene mutant anthocyanless occurred in R_2. It is reasonable to assume that small deficiencies that hardly affect chromosome pairing account for some of the variations.

SUMMARY

1. Treatment with 10,000r X-rays for 33 minutes, or soaking seeds in P^{32} solution, caused formation of unequal pairs, segmental interchange, weakened pairing, inver-

sion, and elongation and stickiness in meiosis of R_1 plants from treated seeds of tomatoes.

2. In R_2 the abnormalities observed in R_1 again occurred, and in addition there were trisomics, deficiency trisomics, homozygous deficients, and duplications.

3. Seedlings in R_1 after P^{32} treatment often had twin or triple shoots and were variegated or occasionally non-pubescent. Most mature plants had relatively normal growth, but some showed somatic changes and consisted of two or more phenotypically and cytologically different parts.

4. In R_2 from P^{32} treatment, a small proportion of seedlings and mature plants were variegated, pale grey-green, or albino. One R_2 family consisted of pale-green plants of reduced fertility, and one slow-growing plant which was a complete mosaic of white, green, glabrous, and hairy tissues. Possibly this may be explained by the irregular distribution of a small fragment.

5. From the X-ray treatment, a mutant resembling wiry *w* was obtained which behaved as a simple recessive.

6. In R_2 from beta radiation of P^{32}, yellow (*r*) and tangerine (*l*) flesh color of fruit gene mutants occurred, also aurea, anthocyanless, male sterile, brachytic, and other mutants of which the genetic nature is unknown.

7. Both X-ray and P^{32} caused much pollen sterility in R_1 and eight R_2 families segregated for complete pollen abortion. Some of the sterility is clearly due to cytological causes; in other cases it seems to be genetic. In most of the other R_2 families fertility was much reduced.

8. An initial dosage of 10.8 microcuries per seed and 5 days' soaking did not prevent germination, but retarded plant growth and drastically affected the plastids, chromosomes, and genes. It probably is close to the lethal dose for tomato seeds.

ACKNOWLEDGMENTS

The authors are indebted to DRS. F. M. TURRELL, J. W. CAMERON, R. K. SOOST, and MR. JOHN WEBER for help in measuring and applying the ionizing agents, and to MR. C. D. MCCARTY for the chromatography.

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A PHENOGENETIC STUDY OF THE LOZENGE PSEUDOALLELES IN DROSOPHILA MELANOGASTER. II. EFFECTS ON THE DEVELOPMENT OF TARSAL CLAWS IN HETEROZYGOTES[1]

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Received January 31, 1956

CONSIDERABLE attention has been drawn to the various cases of apparent multiple alleles which upon critical examination have been found to exh bit a low order of recombination, as if they occupy spatially separable, adjacent gene loci. Such mutants, termed pseudoalleles, exhibit what have been considered to be the physiological criteria of allelism; namely 1) they exhibit similar phenotypic effects, and 2) their heterozygotes exhibit dominance of one or the other member or intermediate phenotypes. In addition, position effects are noted in several cases, as follows: if one considers a pair of pseudoallelic loci, a^+ and b^+, and their respective mutant alleles, a and b, the coupling or *cis* heterozygote, a^+b^+/ab, appears wild type, while the repulsion or *trans* heterozygote, a^+b/ab^+, exhibits the mutant phenotype. Considerable speculation, disagreement, and confusion exists concerning the interpretations to be given to these observations.

The confusion may be avoided if it is recognized that the disagreement is concerned with two quite distinct problems: 1) The observations associated with pseudoallelism have led to interpretations regarding the *structural organization of the hereditary material.*

Three views have been offered: (a) The chromosome is the fundamental unit of heredity. This interpretation proposes that the chromosome is a physical continuum whose intact organization is necessary to exhibit the normal phenotype. Mutations are believed to be due to rearrangements within this organization. Such rearrangements exhibit the separable behavior of genes when subjected to a breeding experiment (GOLDSCHMIDT 1938, 1951). (b) The chromosome consists of a linear arrangement of separable segments. Each segment consists of a grouping of subsegments exhibiting a low order of recombination (PONTECORVO 1953). (c) The genetic material consists of discrete units which are separable on the basis of recombination. This view suggests that pseudoalleles are duplicates which may have arisen by unequal crossing over (LEWIS 1951; GREEN 1955).

2) The second area of disagreement concerns the nature of the *physiological mechanisms* underlying the phenotypic and position effects associated with pseudoallelic loci. (a) One hypothesis proposes that the entire segment acts as a physiological unit. Such regions may exist in alternative forms, subject to both internal

[1] This investigation was supported by research grants G-2278 and G-2440 (C) from the National Cancer Institute of the National Institutes of Health, Public Health Service, and by a grant-in-aid (G-5A) from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

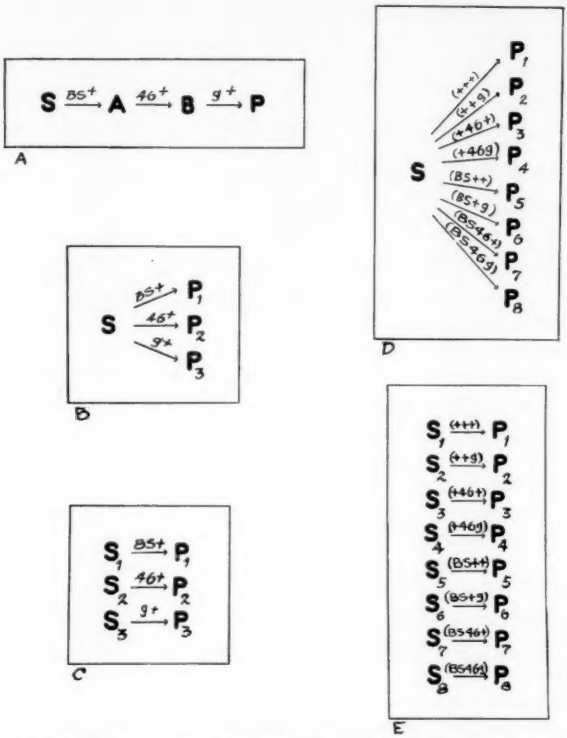

FIGURE 1.—Alternative models for the physiological action of the lozenge pseudoalleles. S is substrate; P is morphogenic product; A and B are intermediates. S_1, S_2, · · · S_n are different, although possibly related, substrates. P_1, P_2, · · · P_n differ either quantitatively or in specificity.

and external recombination, and exhibit all of the physiological properties of alleles (fig. 1, D and E). According to this view, pseudoallelic position effects are merely the reflection of the dominance relations which obtain among physiological alternatives (GOLDSCHMIDT 1951; PONTECORVO 1953). (b) Pseudoallelic loci are concerned with successive steps in a sequence of reactions which may be localized to the site of the genes in the chromosome (fig. 1, A; LEWIS 1951). (c) Pseudoallelic loci are physiological duplicates concerned with the conversion of the same or similar substrate(s) into the same or similar product(s) (fig. 1, B and C; CHOVNICK and FOX 1953).

It should be noted that these positions have been restated to emphasize that they fall into two groups, one concerned with the structure and the other concerned with the function of the hereditary material. When originally elaborated by the various workers concerned, such a separation was not, in fact, maintained. For example, in the development of his views regarding the structure of the hereditary material,

GOLDSCHMIDT draws structural conclusions from physiological observations. On the other hand, GREEN (1955) defines the structural unit in terms of recombination, but maintains that the structural unit is also a functional unit. It has been previously suggested that the investigation of each of these separate problems involves a different set of operations, and that each may be resolved only by evidence obtained from the appropriate operations (CHOVNICK and FOX 1953; BARISH and FOX 1956). The results of the present investigation, a study of the phenotypic effects of pseudoalleles, therefore have direct bearing only upon questions concerning the physiological mechanisms of pseudoallelism. It is recognized that at the primary level of action of the genetic material no such separation can be sensibly maintained; at this level, regardless of the mechanisms involved, structure and function must be intimately correlated. The point to be made is that the congruence of the results of breeding experiments, the principal source along with cytology, of evidence regarding structure, with the results of physiological investigations, the principal source of evidence regarding function, cannot be assumed, and that the operations necessary to identify the primary level of action are not yet available.

The three lozenge pseudoalleles in *Drosophila melanogaster*, which are the subjects of the present work, represent a particularly favorable series for study (GREEN and GREEN 1949). A large number of genotypic combinations are available. Pleiotropy is exhibited, and several variables may be studied. The mutant phenotype includes abnormalities in the external shape and pigmentation of the eye (GOTTSCHEWSKI 1936; OLIVER 1947; GREEN 1948); abnormalities in the various structural components of the ommatidia (CLAYTON 1952); infertility of females associated with abnormalities of genital disc derivatives (OLIVER and GREEN 1944; ANDERSON 1945); antigenic changes (CHOVNICK and FOX 1953); and abnormalities in the structure of the distal segment of the leg, the tarsus (CHOVNICK and LEFKOWITZ 1956).

The latter effects offer, in particular, a system amenable to quantitative evaluation, and exhibit the position effect. The two major structures of the tarsus are the claws and pulvilli. Both structures are affected by the lozenge mutants. The claw

TABLE 1

*Claw size estimates of homozygous genotypes**

Genotype	Mean	Variance	n	$s^2_{\bar{x}}$
+ + +/+ + +	10.34	5.10	91	.056
+ + *g*/+ + *g*	8.91	2.44	162	.015
+ 46 +/+ 46 +	8.25	4.89	143	.034
+ 46 *g*/+ 46 *g*	7.19	5.06	119	.043
BS + +/*BS* + +‡	4.83	6.34	161	.040
BS + *g*/*BS* + *g*‡	4.43	5.48	174	.033
BS 46 +/*BS* 46 +‡	1.07	4.86†	81	.091
BS 46 *g*/*BS* 46 *g*‡	0.00	4.86†	98	.105

* Taken from CHOVNICK and LEFKOWITZ (1956).

† Estimated as the average variance of the other distributions.

‡ Mean, variance, and variance of mean corrected for truncation by the methods of IPSEN (1949).

anomalies range from a reduction in claw pigmentation through reduction in size of claws to complete absence of claws. The effect on the pulvillus, a glandular structure, appears to parallel the claw anomaly. A position effect is evidenced in that coupling heterozygotes are wild type, while repulsion heterozygotes exhibit mutant claws.

All homozygous combinations of wild and mutant pseudoalleles of lozenge (*BS*, 46, and *g*) were previously examined with respect to their effects on the development of the tarsal claws (CHOVNICK and LEFKOWITZ 1956). The mean claw sizes of the various genotypes, expressed in terms of a series of grades, were arrayed in a cumulative fashion (table 1). Depending upon the assumptions made, the observations were consistent with any of the interpretations outlined in figure 1. This being the case, variation in length of tarsal claws associated with positional change of the lozenge pseudoalleles in heterozygotes was studied, and the results are reported in this communication.

MATERIALS AND METHODS

The materials and methods used in this study have been described in the previous report (CHOVNICK and LEFKOWITZ 1956). Eight coisogenic strains differing essentially in the possession of one or another of eight combinations of wild and mutant pseudoalleles of lozenge (*BS*, 46, and *g*) and balanced over a *ClB* chromosome from a single source, were used in matings to produce all offspring described in this report. Observations were restricted to females. Reciprocal crosses were made in each case. No significant differences were found between reciprocal cross offspring, and the means and variances discussed represent pooled results. Several of the distributions exhibited truncation at the lower end of the scale. Correction of mean and variance estimates of such distributions followed the previously described procedure.

RESULTS AND CONCLUSIONS

Dominance effects

The five models portrayed in figure 1 fall into two groups. Three of the models (A, B, and C) are based on the suggestion that the action of each of the lozenge loci is independent of the other two, except as they may be related with respect to substrate specificity. The remaining models (D and E) are based on the supposition that the entire lozenge segment, including all three loci, acts as a single unit of action. An attempt to distinguish between these two alternative groups may be made by examining the dominance effects exhibited in the present data.

If one were to assume that the unit of physiological action with respect to tarsal development is the entire lozenge segment, then the various combinations of wild and mutant pseudoalleles would comprise the alternative forms of this unit. Each alternative form, according to this view, is designated by a combination of wild type and mutant alleles at three points in the segment. Eight alternative forms are so designated, and these may be arranged in the following serial order, according to the degree of their effects in homozygotes: + + +, + + *g*, + 46 +, + 46 *g*, *BS* + +, *BS* + *g*, *BS* 46 +, *BS* 46 *g* (table 1). If this view is correct, examination of dominance effects in heterozygotes involving all combinations of these eight al-

TABLE 2

Claw size estimates of heterozygous genotypes

Genotype	Mean	Variance	n	$\frac{s^2}{n}$
+ + g/+ 46 +	9.18	2.41	154	0.016
+ + g/+ 46 g	9.11	3.82	82	0.047
+ + g/*BS* + +	8.77	2.82	102	0.028
+ + g/*BS* + g	8.75	2.81	94	0.030
+ + g/*BS* 46 +	8.97	3.62	120	0.030
+ + g/*BS* 46 g	8.85	3.16	131	0.024
+ 46 +/+ 46 g	8.08	5.96	143	0.042
+ 46 +/*BS* + +	8.01	3.44	95	0.036
+ 46 +/*BS* + g	7.18	4.10	108	0.038
+ 46 +/*BS* 46 +	7.04	4.49	108	0.042
+ 46 +/*BS* 46 g	6.56	4.29	140	0.031
+ 46 g/*BS* + +	7.01	6.73	112	0.060
+ 46 g/*BS* + g	7.09	4.77	143	0.033
+ 46 g/*BS* 46 +	5.38	4.05	119	0.034
+ 46 g/*BS* 46 g	5.10	2.99	98	0.031
BS + +/*BS* + g*	4.70	3.91	163	0.025
BS + +/*BS* 46 +*	3.21	3.51	119	0.032
BS + +/*BS* 46 g*	1.75	5.41	112	0.072
BS + g/*BS* 46 +*	3.23	2.71	128	0.022
BS + g/*BS* 46 g*	1.58	6.41	109	0.094
BS 46 +/*BS* 46 g*	−2.62	4.09†	83	0.187

* Mean, variance, and variance of mean corrected for truncation by the methods of IPSEN (1949).

† Estimated as the average variance of the other distributions.

ternatives would reveal a pattern resembling those commonly associated with allelic interaction.

All heterozygotes possessing one chromosome carrying + + + lozenge region have been reported to exhibit the wild phenotype (GREEN and GREEN 1949). Our observations support this conclusion. They have large, pigmented claws, well developed pulvilli, and wild type eyes. Thus, the highest member of the above series is completely dominant over all other members of the series. All other combinations of the various alternatives exhibit mutant phenotypes, but an examination of the data discloses dominance in several cases. Thus, the next member of the series, + + *g*, exhibits complete dominance over all lower members of the series (table 2). The + 46 + segment exhibits complete dominance over the next two combinations, + 46 *g* and *BS* + +, but shows intermediate effects with lower combinations in the series (table 2). Similarly, the + 46 *g* segment exhibits complete dominance over the next two, *BS* + + and *BS* + *g*, and intermediate effects with lower combinations. All remaining combinations show intermediate effects. Clearly, the interaction

pattern is reminiscent of allelic interaction. With respect to dominance relationships, therefore, these data are consistent with the view that the entire segment operates as a physiological unit.

By way of contrast, an attempt to interpret these data in terms of the hypothesis that each of the lozenge loci is an independently operating physiological unit leads to inconsistencies. In heterozygotes involving a + + + segment, all three mutants are completely recessive to their respective wild alleles. When, however, + + g/ + + *g* is compared with the remaining heterozygotes possessing a + + *g* segment (table 2), *g* appears to be dominant to g^+. Similar difficulties are encountered with respect to *BS* and 46. Such inconsistencies should be called position effects, but to do so would impose an additional problem upon those which already exist.

The simplest conclusion to be drawn is that the lozenge loci do not function as independent physiological units, clearly ruling out the hypothesis represented in figure 1C. Kinetic considerations (WRIGHT 1941; STRAUSS 1955), relating to substrate utilization, might be developed to reconcile the hypotheses portrayed in A and B with the data, but only at the expense of considerable complexity. It would seem simpler to conclude that the entire segment operates as an integrated unit in tarsal development (fig. 1, D and E). Each of the alternative segments, as specified by a particular combination of wild and mutant alleles at three points, exhibits a unified function.

As pointed out by GOLDSCHMIDT (1951), this view possesses the added advantage of dissolving the problems posed by the apparent position effects exhibited by pseudoalleles. Such effects are now seen as reflections of the dominance relationships which exist among the segmental alternatives. No special hypothesis is needed to explain them, since they are to be expected under the conventional views regarding dominance.

Mode of action of the lozenge segments

Accepting the conclusion that the lozenge segment acts as a physiological unit, it now becomes possible to inquire into the mode of action of the various alternatives with respect to each other in individuals possessing two X chromosomes. This question is most easily examined in those cases not obscured by dominance. More specifically, in the case of those segments which do not exhibit dominance, do the segments present in such individuals act in an additive fashion on the chosen scale of measurement, or are they non-additive in effect?

The model which has been proposed (fig. 1, D and E) assumes that each lozenge segment mediates the rate of conversion of a substrate into a morphogenic product which quantitatively determines claw size. The truncation at the lower end of the scale, and cumulative effects in homozygotes, previously led to the following view: (1) A minimum quantity of product is necessary before a claw can be produced. (2) After this threshold concentration is reached, claw size is directly related to the amount of product available. By correcting for the threshold at the lower end of the scale, the mean claw sizes of the homozygous genotypes may be taken as estimates of the amount of morphogenic product attributed to the genotype (CHOVNICK and LEFKOWITZ 1956). According to this view, each of the lozenge segments is

concerned with the amount of morphogenic product made available during the critical point of tarsal development.

The hypothesis of additive action of the segments may be subjected to test by using it to predict mean claw sizes for the various heterozygotes from the observed means of the homozygotes. Assuming that the lozenge segments operate additively, the mean claw size of any homozygous genotype represents an estimate of twice the morphogenic product contribution attributable to one segment. Thus, $P_{BS++} = \frac{1}{2}\bar{x}_{BS++/BS++}$, and this value may be used as an estimate of the morphogenic product contribution of one X chromosome carrying the combination of lozenge pseudoalleles *BS* + +. In this fashion, estimates may be generated for chromosomes bearing all combinations of wild and mutant pseudoalleles of lozenge which do not exhibit dominance. Prediction of the mean claw size for any heterozygous genotype is achieved as follows:

$$\bar{x}_{BS++/BS\,46+} = P_{BS++/BS\,46+} = P_{BS++} + P_{BS\,46+} \tag{1}$$

and

$$\bar{x}_{BS++/BS\,46+} = \tfrac{1}{2}\bar{x}_{BS++/BS++} + \tfrac{1}{2}\bar{x}_{BS\,46+/BS\,46+} \tag{2}$$

Comparison of predicted and observed values is made by examination of the test statistic, *c*, obtained as the ratio of the difference between observed and expected values to the standard error of the difference. For the genotype *BS* + +/*BS* 46 +, the test statistic *c*, may be obtained as the absolute value of (3).

$$\frac{\bar{x}_{BS++/BS\,46+} - \tfrac{1}{2}\bar{x}_{BS++/BS++} - \tfrac{1}{2}\bar{x}_{BS\,46+/BS\,46+}}{\sqrt{s^2_{\bar{x}_{BS++/BS\,46+}} + \tfrac{1}{4}s^2_{\bar{x}_{BS++/BS++}} + \tfrac{1}{4}s^2_{\bar{x}_{BS\,46+/BS\,46+}}}} \tag{3}$$

Since the dominance effects preclude accurate estimation of the contribution of segments + + +, + + *g*, + 46 *g* by this method, heterozygotes involving these segments are omitted from consideration. The remaining genotypes may be tested, and are included in the upper portion of table 5. With one exception, all of the means observed do not differ significantly from the values expected on the hypothesis of additive action of lozenge segments. The one exceptional case is that of *BS* 46 +/*BS* 46 + and *BS* 46 *g*/*BS* 46 *g*. Examination of the distribution of claw values for these genotypes reveals that very few flies of these groups possess claws (CHOVNICK and LEFKOWITZ 1956). Since that portion of the distribution above the truncation point represents so few flies, small sampling fluctuations would have profound effect upon the estimate of the mean adjusted for truncation. Moreover, the observed distribution of the heterozygote *BS* 46 +/*BS* 46 *g* falls in this same category. These three genotypes had the lowest means in the entire study. For these cases, the estimate of variance was taken as the average variance of the other distributions, and the error incurred in the estimation of adjusted means is not afforded by the sampling variance estimates.

It is concluded that the lozenge segments carry out their functions in an additive fashion on the chosen scale. This conclusion stands in contrast to that previously reached with respect to the individual lozenge loci, whose effects in homozygotes were observed not to be additive on the same scale (CHOVNICK and LEFKOWITZ

1956). The hypothesis that the lozenge segments as a whole operate as physiological units in tarsal development is thus strengthened.

Additive action of lozenge segments and dominance effects

The preceding analysis omits consideration of those segments which exhibit dominance. Dominance effects in mutant heterozygotes, however, must be considered in any interpretation of the mode of action of lozenge in tarsal development. The model which has been proposed (fig. 1, D and E) assumes that each lozenge segment mediates the rate of conversion of a substrate into a product which quantitatively determines claw size. As demonstrated in the previous section, such action, in heterozygotes which do not exhibit dominance, is additive on the chosen scale.

The observation of dominance effects leads to an extension of this interpretation. If additivity is assumed to be characteristic of even those segments which exhibit dominance, it is possible to develop a consistent interpretation from the considerations summarized graphically in figure 2. The horizontal axis in this figure represents the scale of variation in morphogenic product made available by the genotype. Within any one genotype, individual variation in amount of this product is attributable to environmental sources. T_0 represents the threshold below which the amount of product is insufficient to result in claw development. The location of the T_0 threshold is described in a previous report (CHOVNICK and LEFKOWITZ 1956).

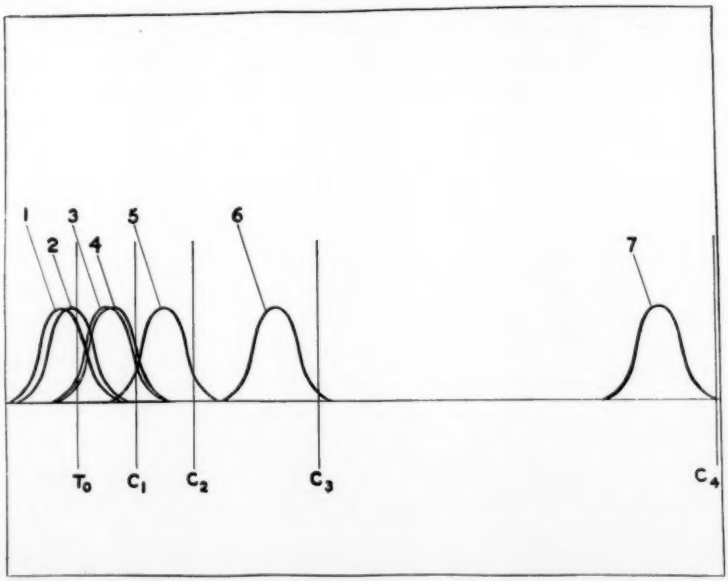

FIGURE 2.—Diagrammatic representation of the amount of morphogenic product conditioned by lozenge genotypes, plotted on an additive scale. Position of threshold and ceilings indicated. (1) *BS* 46 *g*/*BS* 46 *g*, (2) *BS* 46 +/*BS* 46 +, (3) *BS* + *g*/*BS* + *g*, (4) *BS* + +/*BS* + +, (5) + 46 *g*/+ 46 *g*, (6) + 46 +/+ 46 +, (7) + + *g*/+ + *g*.

The observed distributions of claw sizes for the homozygous genotypes *BS* 46 *g*/*BS* 46 *g* and *BS* 46 +/*BS* 46 + indicate that the mean amount of morphogenic product for each falls below the T_0 threshold (table 1). Similarly, the mean amount of morphogenic product attributable to each of the homozygous genotypes *BS* + *g*/*BS* + *g* and *BS* + +/*BS* + + would be located to the right of the T_0 threshold. Numerical values could be assigned to the mean amounts of morphogenic product corresponding to the mean claw size estimates for the various genotypes: *BS* 46 *g*/*BS* 46 *g*, 0.00; *BS* 46 +/*BS* 46 +, 1.07; T_0 threshold, 1.56; *BS* + *g*/*BS* + *g*, 4.43; *BS* + +/*BS* + +, 4.83.

The four chromosomal segments thus far considered do not exhibit dominance. The observed mean claw sizes of the various heterozygous combinations of these segments would therefore correspond to the amount of product expected if these segments operate in an additive fashion, as demonstrated in the previous section. Thus, the mean amount of product for each of these heterozygous combinations could be plotted on the same scale as that already used for the homozygotes. While they have been omitted from the diagram for purposes of simplicity, their values are as follows (predicted means from table 5): *BS* 46 +/*BS* 46 *g*, 0.54; *BS* + *g*/*BS* 46 *g*, 2.22; *BS* + +/*BS* 46 *g*, 2.42; *BS* + *g*/*BS* 46 +, 2.75; *BS* + +/*BS* 46 +, 2.95; *BS* + +/*BS* + *g*, 4.63.

If mean claw size is always a direct measure of the amount of morphogenic product made available by the genotype, the next homozygous genotype, + 46 *g*/+ 46 *g*, would be located at 7.19 on this same scale. Furthermore, if this segment operates in an additive fashion in heterozygotes with the previous segments, the mean amount of morphogenic product in each heterozygote should be intermediate between the corresponding homozygotes. The observed claw sizes of such heterozygotes would then be expected to exhibit intermediate values. In point of fact, the + 46 *g* segment exhibits dominance over the *BS* + + and *BS* + *g* segments; i.e., the observed mean claw values are not different from that of the + 46 *g*/+ 46 *g* homozygote. If it is nevertheless assumed that additivity still operates, these observations must mean that a ceiling exists, C_1, which is subject to at least two possible interpretations: (1) Further increase in amount of morphogenic product takes place after the critical period for tarsal development, and thus leads to no increase in claw size. (2) Substrate limitation prevents production of morphogenic product over this ceiling. Under either circumstance, the genotype + 46 *g*/+ 46 *g* must be capable of conditioning the production of an amount of morphogenic product well over this ceiling, since one dose of + 46 *g* is sufficient to carry heterozygotes with *BS* + + or *BS* + *g* over the ceiling.

Since the C_1 ceiling is that point at which further increase in morphogenic product leads to no further increase in claw size, this point must be located at 7.19 on the scale. It is then possible to estimate $P_{+\ 46\ g}$, the morphogenic product contribution of one dose of + 46 *g*, in the following manner. If one dose of *BS* + *g* contributes 2.22 units of morphogenic product, i.e., one half of the mean claw value of *BS* + *g*/*BS* + *g*, then one dose of + 46 *g* must contribute at least 4.97 such units if the mean product value of + 46 *g*/*BS* + *g* is to exceed the C_1 ceiling. Thus the homozygote + 46 *g*/+ 46 *g* must be capable of producing an amount of morphogenic product no less than 9.94 units on the scale (2 × 4.97).

As a check on this interpretation, it is possible to predict the amounts of morphogenic product expected in the heterozygotes + 46 *g*/*BS* 46 + and + 46 *g*/*BS* 46 *g*. Such predictions would be based upon the additivity of the *BS* 46 + and *BS* 46 *g* contributions as estimated in the previous section, with the + 46 *g* contribution as estimated in the previous paragraph. For the + 46 *g*/*BS* 46 + genotype, the predicted amount of morphogenic product would be 5.51 (4.97 + 0.54). For the + 46 *g*/*BS* 46 *g* heterozygote, the predicted amount of morphogenic product would be 4.97 (4.97 + 0.00). Since these values fall below the C_1 level, they would be expected to correspond to the observed claw values. This expectation is fulfilled (table 5).

For those genotypes which fall below a ceiling, comparison of expected and observed claw values is accomplished by examination of the test statistic, *c*. Computation of *c* is modified from that presented in the previous section to account for the existence of the ceiling. Thus, for the heterozygote, *BS* 46 *g*/+ 46 *g*, the expected amount of morphogenic product $P_{BS\ 46\ g/+\ 46\ g} = P_{BS\ 46\ g} + P_{+\ 46\ g}$. For the segment, *BS* 46 *g*, which does not exhibit ceiling effects, $P_{BS\ 46\ g} = \frac{1}{2}\bar{x}_{BS\ 46\ g/BS\ 46\ g}$. However, for the segment, + 46 *g* which does exhibit a ceiling,

$$P_{+\ 46\ g} = \bar{x}_{+\ 46\ g/+\ 46\ g} - \tfrac{1}{2}\bar{x}_{BS\ +\ g/BS\ +\ g}. \tag{4}$$

Thus,

$$P_{BS\ 46\ g/+\ 46\ g} = \tfrac{1}{2}\bar{x}_{BS\ 46\ g/BS\ 46\ g} + \bar{x}_{+\ 46\ g/+\ 46\ g} - \tfrac{1}{2}\bar{x}_{BS\ +\ g/BS\ +\ g}. \tag{5}$$

Since the estimate, $P_{BS\ 46\ g/+\ 46\ g}$, falls below the C_1 ceiling, then

$$P_{BS\ 46\ g/+\ 46\ g} = \bar{x}_{BS\ 46\ g/+\ 46\ g} = \tfrac{1}{2}\bar{x}_{BS\ 46\ g/BS\ 46\ g} + \bar{x}_{+\ 46\ g/+\ 46\ g} - \tfrac{1}{2}\bar{x}_{BS\ +\ g/BS\ +\ g}. \tag{6}$$

The test statistic, *c*, then takes the form of (7).

$$\frac{\bar{x}_{BS\ 46\ g/+\ 46\ g} - \tfrac{1}{2}\bar{x}_{BS\ 46\ g/BS\ 46\ g} - \bar{x}_{+\ 46\ g/+\ 46\ g} + \tfrac{1}{2}\bar{x}_{BS\ +\ g/BS\ +\ g}}{\sqrt{s^2_{\bar{x}_{BS\ 46\ g/+\ 46\ g}} + \tfrac{1}{4}s^2_{\bar{x}_{BS\ 46\ g/BS\ 46\ g}} + s^2_{\bar{x}_{+\ 46\ g/+\ 46\ g}} + \tfrac{1}{4}s^2_{\bar{x}_{BS\ +\ g/BS+\ g}}}} \tag{7}$$

Continued treatment of the remaining genotypes along this line provides complete accounting for their phenotypes. This treatment requires the assumption of additional ceilings in order to account for the various dominance effects (C_2, C_3, C_4), but additivity of the contributions of the various segments to the amount of morphogenic product in each genotype extends over the whole scale. Further evidence that this is the case is obtained from the observation that the observed variance of each genotype is in no way correlated with its mean claw size (tables 1 and 2). The position of each ceiling and the manner in which it was estimated is given in table 3. The contribution of morphogenic product of each of the eight lozenge segments and the source of its estimation is given in table 4. Table 5 contains the following items: the predicted amounts of morphogenic product (*P*) conditioned by all genotypes, calculated in the manner indicated above; the expected claw value for each genotype, taking into consideration ceiling effects; the observed claw values for all genotypes; and the test statistic, *c*, for the differences between expected and observed

TABLE 3

Scale position of ceilings

Ceiling	Scale position	Source of estimate
C_1	7.19	+ 46 *g*/+ 46 *g*
C_2	8.25	+ 46 +/+ 46 +
C_3	8.91	+ + *g*/+ + *g*
C_4	10.34	+ + +/+ + +

TABLE 4

Morphogenic product contributed by lozenge segments

Segment	Contribution	Source of estimate
+ + +	10.34	+ + +/+ + +, because of complete dominance
+ + *g*	8.91	+ + *g*/+ + *g*, because of complete dominance
+ 46 +	5.83	$C_2 - (BS + +)$
+ 46 *g*	4.97	$C_1 - (BS + g)$
BS + +	2.42	$\frac{1}{2}$ (*BS* + +/*BS* + +)
BS + *g*	2.22	$\frac{1}{2}$ (*BS* + *g*/*BS* + *g*)
BS 46 +	0.54	$\frac{1}{2}$ (*BS* 46 +/*BS* 46 +)
BS 46 *g*	0.00	$\frac{1}{2}$ (*BS* 46 *g*/*BS* 46 *g*)

claw values. Calculation of c is not justified for those genotypes which served as sources of information for the estimation of ceiling positions or segmental contributions.

For those heterozygotes with predicted amounts of morphogenic product (P) which fall at or above ceiling levels, the expected claw values are estimated to be equal to that of the homozygote in the series. That this is indeed the case may be seen from comparison of the heterozygote samples with the homozygote by test of the hypothesis that they are drawn from the same population. Such a test takes the form of an analysis of variance and values of F are indicated in table 5.

In table 5, the 36 lozenge genotypes have been arranged in five groups. The first group contains the genotypes for the lowest segments in the series, i.e. those exhibiting no dominance. In this group, a linear relationship exists between the predicted amount of morphogenic product (P) and the observed claw values (fig. 3). The second group contains the + 46 *g*/+ 46 *g* homozygote, and heterozygotes of + 46 *g* with lower members of the segmental series. Here a linear relationship between P and observed claw values is exhibited up to the C_1 ceiling, where an abrupt change of slope occurs so that the observed claw values never exceed that ceiling (7.19). Indeed, these first two groups could be combined into a single group exhibiting a linear relationship between P and observed claw values up to C_1, where an abrupt change in slope would occur. The third group of genotypes in table 5 are those involving + 46 +, homozygous or heterozygous with lower members of the series. Here again, a linear relationship between P and observed claw values is exhibited up to the C_2 ceiling, where an abrupt change of slope occurs so that the observed claw values never exceed that ceiling (8.25). All genotypes involving + +

TABLE 5

Morphogenic product, and estimated and observed claw values of lozenge genotypes

Genotype	*P*	Claw values		*c**	*F*
		Expected	Observed		
BS 46 *g*/*BS* 46 *g*	0.00	0.00	0.00		
BS 46 *g*/*BS* 46 +	0.54	0.54	−2.62	6.49	
BS 46 +/*BS* 46 +	1.07	1.07	1.07		
BS 46 *g*/*BS* + *g*	2.22	2.22	1.58	1.73	
BS 46 *g*/*BS* + +	2.41	2.41	1.75	2.02	
BS 46 +/*BS* + *g*	2.75	2.75	3.23	2.09	
BS 46 +/*BS* + +	2.95	2.95	3.21	1.12	
BS + *g*/*BS* + *g*	4.43	4.43	4.43		
BS + *g*/*BS* + +	4.63	4.63	4.70	0.33	
BS + +/*BS* + +	4.83	4.83	4.83		
BS 46 *g*/+ 46 *g*	4.97	4.97	5.10	0.40	
BS 46 +/+ 46 *g*	5.51	5.51	5.38	0.43	
BS + *g*/+ 46 *g*	7.19	7.19	7.09		$F = 0.183$
BS + +/+ 46 *g*	7.38	7.19	7.01		*d.f.* = 2, 371
+ 46 *g*/+ 46 *g*	9.94	7.19	7.19		
BS 46 *g*/+ 46 +	5.83	5.83	6.56	2.30	
BS 46 +/+ 46 +	6.37	6.37	7.04	2.03	
BS + g/+ 46 +	8.05	8.05	7.18	2.90	
BS + +/+ 46 +	8.25	8.25	8.01		$F = 0.913$
+ 46 *g*/+ 46 +	10.80	8.25	8.08		*d.f.* = 2, 378
+ 46 +/+ 46 +	11.66	8.25	8.25		
BS 46 *g*/+ + *g*	8.91	8.91	8.85		
BS 46 +/+ + *g*	9.45	8.91	8.97		
BS + *g*/+ + *g*	11.13	8.91	8.75		
BS + +/+ + *g*	11.33	8.91	8.77		
+ 46 *g*/+ + *g*	13.88	8.91	9.11		$F = 0.415$
+ 46 +/+ + *g*	14.74	8.91	9.18		*d.f.* = 6, 838
+ + *g*/+ + *g*	17.82	8.91	8.91		
BS 46 *g*/+ + +	10.34	10.34			
BS 46 +/+ + +	10.88	10.34			
BS + *g*/+ + +	12.56	10.34			
BS + +/+ + +	12.76	10.34			
+ 46 *g*/+ + +	15.31	10.34			
+ 46 +/+ + +	16.17	10.34			
+ + *g*/+ + +	19.25	10.34			
+ + +/+ + +	20.68	10.34	10.34		

* $P\ (c \geq 2.58) = .01$.

g are associated with *P* values exceeding the C_3 ceiling, and observed claw values do not deviate significantly from 8.91, the value of C_3. All genotypes involving + + + exhibit a similar relationship to C_4. Observed means for the heterozygotes involving + + + are not included in table 5. Dominance of the + + + segment over all

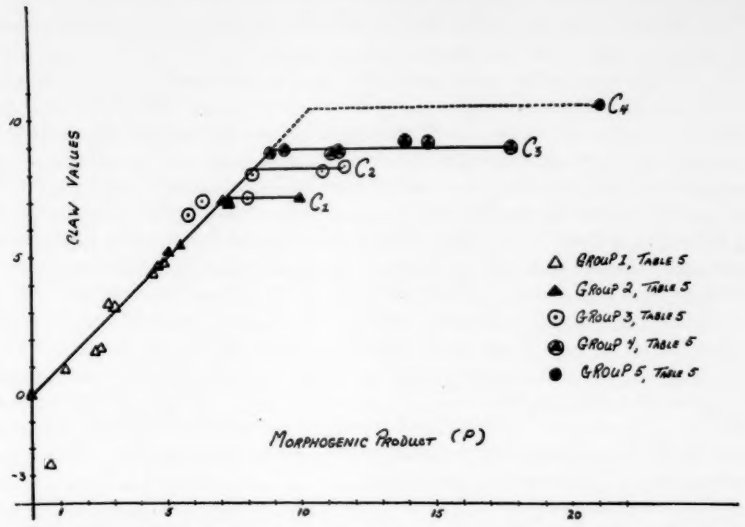

FIGURE 3.—Relationship between observed claw value and predicted morphogenic product for all homozygous and heterozygous lozenge genotypes.

lower members of the series had been reported previously (GREEN and GREEN 1949). Examination of small samples of these heterozygotes supported this view, and a detailed analysis of this group of heterozyogtes was omitted.

As WRIGHT (1941) has demonstrated on the basis of enzyme kinetics, allelic series are expected to exhibit relationships such as are observed for the lozenge segmental alternatives if the effects are enzymatically mediated as in the following model:

gene or segment
↓
enzyme
↓
$S \rightarrow P \rightarrow$ Observed phenotype

In terms of this model, the following assumptions have been made in the calculation of predicted amounts of morphogenic product (P): 1) the existence of a steady state in the conversion of S to P over a specific interval of time during development, 2) the production by the alternative segments of differing amounts of enzyme rather than of enzymes differing in specificity, and 3) strict proportionality of the rate of production of P to concentration of enzyme. Under these circumstances, the predicted amounts of morphogenic product may be equated to the concentration of enzyme conditioned by each alternative segment, and the observed claw values may be equated to the rate of conversion of S to P. Figure 3 could therefore be taken as portraying the relationship between enzyme concentration and the rate of conversion

of S to P, and a departure from linearity in this relationship could result from departures from any of the assumptions listed above.

Slight differences in the specificity of the enzyme produced by alternative segments could result in differences in its Michaelis constant (K_s). WRIGHT (1941) has examined two limiting cases of such changes, i.e. when K_s becomes very large (approaching ∞) and when K_s becomes very small (approaching 0). In both cases, a ceiling in the rate of production of P results from substrate limitation. In the former case (K_s very large), the approach to the ceiling is asymptotic, while in the latter (K_s very small) it is linear. The data for Groups 1 and 2 (fig. 3) appear to approximate those expected when K_s is very small, but those for the other groups are insufficiently extensive to allow a decision as to whether changes of this sort are involved.

Alternatively, slight differences in enzyme specificity could result in the production from a single substrate of morphogenic products which differ in their specificity for claw production (fig. 1D). A ceiling to the rates of the alternative reactions would be imposed by substrate limitation, and would be independent of the K_s of the alternative enzymes, but at the phenotypic level several different ceilings could result from the differing specificities of the alternative morphogenic products. This could give rise to a situation such as is observed.

Larger differences in enzyme specificity could result in the utilization of different substrates by the different lozenge segments (fig. 1E). In each case a ceiling would arise from substrate limitation, but the level of the ceiling would vary from substrate to substrate. A situation such as that observed would readily result.

Finally, substitution of the alternative segments might result in changes of a different sort, i.e. in the duration of the interval of time during which S is converted into P. The multiple ceilings would then reflect time limitations to the production of morphogenic product, the specific details of which could be fitted to the observed results.

It thus becomes impossible to distinguish between figures 1D and 1E, or to specify further detail on the basis of these data. Indeed, the actual state of affairs may well consist of a combination of the conditions just discussed.

DISCUSSION

While the present data are most simply and consistently explained by the hypothesis that the entire lozenge segment acts as a physiological unit, it is not necessary to suppose that this is the only physiological mechanism associated with pseudoallelism. In the case of the vermilion pseudoalleles, the point has already been made that the entire segment appears to act as a physiological unit at one phenotypic level, but that at a second level the individual loci exhibit physiological unity, while at still another level each locus appears to consist of two or more physiological units (BARISH and FOX 1956). We would venture to suggest that each case of pseudoallelism deserves separate analysis and might well exhibit unique physiological properties.

From the most general point of view, however, the hypothesis that the entire pseudoalleic segment operates as a physiological unit would seem to bring the greatest degree of order to the existing data. In the first place, no physiological properties

need be assumed other than those usually attributed to alleles. Secondly, pseudoallelic position effects are dismissed as special problems, and become simply reflections of dominance relationships. In this connection, a more special difficulty is also dispelled, namely, the apparent contradiction between the position effects exhibited by the lozenge and vermilion pseudoalleles on morphological traits and their lack of position effects on antigenic specificity (CHOVNICK and FOX 1953; BARISH and FOX 1956). This disparity may now be seen as another example of differing dominance relationships among pleiotropic effects of particular genes. A further special problem which is greatly simplified by this point of view is that posed by the relationships of the vermilion mutants to their non-allelic suppressor (GREEN 1954, 1955). The complexity of these relationships (BARISH and FOX 1956) becomes easily explicable if the unit whose physiological action is affected by the suppressor is the entire vermilion segment. Finally, the so-called "transvection effects" which have been reported in the bithorax case (LEWIS 1954a, 1954b, 1955) become, on this view, true position effects resulting from rearrangement, rather than special physiological consequences of pseudoallelism.

From one point of view, the hypothesis represented in figure 1A might be considered a special case of the view just presented, i.e., as a particular mechanism by means of which a pseudoallelic segment could exhibit an apparently unified action. Such a view would require the following assumptions: 1) a linear sequence of reactions; 2) that this sequence is localized to the immediate vicinity of the chromosome; 3) that each locus in the segment is a discrete functional unit; 4) that each step in the sequence is controlled by one locus. Aside from the fact that evidence is available that suggests interchromosomal interaction in certain cases (CHOVNICK and FOX 1953; LEWIS 1954b and 1955) and non-linearity in others (BARISH and FOX 1956), the complexity of this view is not conducive to its acceptance.

It should be repeated that from an operational point of view, the conclusion that pseudoallelic segments may act as integrated physiological units has no necessary implications for questions related to the structural organization of the hereditary material. Discussion of the evidences which do bear on this question would be inappropriate in the present paper.

SUMMARY

In a study of the physiology of psueodallelism, the effects on the development of tarsal claws of a series of eight different lozenge segments (+ + +, + + *g*, + 46 +, + 46 *g*, *BS* + +, *BS* + *g*, *BS* 46 +, *BS* 46 *g*) have been examined in all heterozygous combinations. The interaction effects in heterozygotes lead to the conclusion that the entire lozenge segment operates as an integrated physiological unit. The pseudoallelic position effects are seen as reflections of the dominance relations which exist among the segmental alternatives. Additivity of the action of the lozenge segments is indicated. Threshold and ceiling effects suggest several alternative schemes of action of the various lozenge segments. In all cases, the various alternative lozenge segments are concerned with the production of morphogenic products which may differ in specificity, but which exert quantitative effects on tarsal development by affecting a common developmental process.

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MUTAGENESIS IN MATURE DROSOPHILA SPERMATOZOA BY "TRIAZINE" APPLIED IN VAGINAL DOUCHES[1]

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Received February 8, 1956

MUTAGENESIS in germ cells of *Drosophila melanogaster* males by 2:4:6-tri (ethyleneimino)-1:3:5-triazine ("triazine") has been proven and investigated by the FAHMY's (BIRD 1952; FAHMY and BIRD 1953; FAHMY and FAHMY 1953, 1954, 1955) and has also been studied by LÜERS (1953). The purposes of the present studies were to test whether triazine, like nitrogen mustard (HERSKOWITZ 1947, 1949, 1955a; SCHALET and HERSKOWITZ 1954; SCHALET 1955), formaldehyde (HERSKOWITZ 1953, 1955a), propylene oxide and ethyl sulfate (SCHALET 1954) is mutagenic to sperm known to be mature at the time of treatment, and if so (a) whether it is mutagenically more effective to treat mature sperm by means of a succession of vaginal douches of a weaker solution of triazine rather than by a single treatment of a higher concentration, and (b) whether triazine can also induce a relatively high frequency of reciprocal translocations under the same conditions that gave a positive response for a nitrogen mustard (SCHALET 1955). Some of the results presented have been reported earlier (HERSKOWITZ 1955b).

MATERIAL AND METHODS

Drosophila melanogaster were used in these experiments. Females which had been inseminated, by being placed with males of appropriate genetic constitution a day earlier, were treated with saline solutions (prepared according to BUCK and MELLAND 1942) of approximately 0.1, 0.2, and 1.0% triazine administered in vaginal douches (HERSKOWITZ 1955a). Treated females were isolated individually in vials containing culture medium, and were transferred to new food-containing vials four times at 3-day intervals to furnish five successive broods (except that the second brood after the 0.2% triazine treatments lasted only one day).

Females were treated with 1.0% triazine only once but some treated with 0.2% triazine were treated again with 0.2% solution after four days (at the end of brood 2) and some of these were treated again with 0.2% solution after three additional days (at the end of brood 3).

Tests were made to detect several different types of mutation in sperm-contributed chromosomes employing MULLER's (1954) standard "multi" technique: complete or certain partial losses of either paternal sex chromosome as determined from examination of the F_1 males, translocations and X chromosome recessive lethal mutations as

[1] These experiments have been supported by a grant received for work of H. J. MULLER and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.

[2] Contribution No. 617.

determined from the offspring of appropriate crosses of the F_1 male and female, respectively. For 0.1% treatments only sex chromosome losses were studied.

All work was done at 25 ± 1°C.

TABLE 1

Fate of females treated with triazine in postcopulatory vaginal douches

Conc., %	No. ♀♀			
	Treated	Dead in 48 hrs.	Ovipositing	Producing adult offspring
0.1	68	0	62	60
0.2 once	184	8	174	159
0.2 twice	98	—	—	86
0.2 thrice	48	—	—	34
1.0	197	22	169	135

TABLE 2

Percent sex chromosome losses following treatment of sperm, in the females listed in table 1. (No. mutations/no. tests is given in parentheses.)

Conc., %	Broods in which sperm were used			
	1 + 2	3	4 + 5	ALL
0.1	0.13 (4/3118)	0.12 (1/836)	0.20 (3/1530)	0.15 (8/5484)
0.2 once	0.14 (3/2174)	0.28 (2/713)	0.86 (5/579)	0.29 (10/3466)
0.2 twice	—	0.35 (4/1152)	0.5 (2/427)	0.38 (6/1579)
0.2 thrice	—	—	0.4 (1/272)	0.4 (1/272)
1.0	0.4 (3/747)	0.6 (5/854)	0.4 (3/733)	0.47 (11/2334)

TABLE 3

Percent translocations (T) and sex-linked recessive lethals (L) following treatment of sperm, in the females listed in table 1. Typical control rates for (L) and (T) are approximately 0.2% and 0.0%, respectively. (No. tests is given in parentheses.)

Conc., %		Broods in which sperm were used			
		1 + 2	3	4 + 5	ALL
0.2	T	0.7 (876)	1.9 (453)	0.8 (236)	1.1 (1565)
once	L	2.5 (1171)	5.4 (654)	4.3 (282)	3.7 (2107)
	T/L	0.31	0.35	0.19	0.30
0.2	T	—	1.2 (583)	3.8 (317)	2.1 (905)
twice	L	—	6.2 (803)	5.9 (340)	6.1 (1173)
	T/L	—	0.19	0.64	0.34
0.2	T	—	—	5.0 (198)	5.0 (198)
thrice	L	—	—	6.2 (243)	6.2 (243)
	T/L	—	—	0.81	0.81
1.0	T	0.4 (532)	2.6 (573)	6.1 (545)	3.0 (1650)
	L	6.8 (648)	6.8 (784)	6.9 (623)	6.8 (2055)
	T/L	0.06	0.38	0.88	0.44

RESULTS

The fate of 449 females treated with solutions of triazine administered in postcopulatory vaginal douches is summarized in table 1. The frequencies of sex chromosome losses, as determined from the phenotype of the F_1 sons of the survivors of these treatments, are given in table 2 for different treatments and for different broods. Table 3 gives the results of tests for different treatments and for different broods for translocations and for X-linked recessive lethal mutations, as well as the values obtained by dividing the translocation by the lethal frequencies. There was a total of 70 II-III, 15 Y-II, and 11 Y-III reciprocal translocations obtained following treatment.

DISCUSSION

The results in table 2 show that while the rates for sex chromosome losses obtained in different broods after treatment with 0.1% triazine were no different from the control mutation rate of 0.16% obtained earlier in comparable extensive work of MULLER, HERSKOWITZ and OSTER (unpublished), the overall rate of 0.47% for the 1.0% treatments is significantly higher than the overall rate of 0.15% for the 0.1% treatments, the treatments with dosages between these extremes having intermediate mutation rates. This establishes that triazine induces sex chromosome losses in mature sperm.

The mutagenic effect of treating inseminated females one, two or three times with 0.2% triazine solutions can be compared by examining the data of broods 4 + 5 for the different treatments. While these data for sex chromosome losses (table 2) actually go in the direction opposite from expectation, the sample sizes are so small and the number of losses so few that the statistical errors are large enough to hide a considerable tendency even in the expected direction. In table 3, however, there is an increase in both translocations and lethals with the number of treatments, the translocation frequencies for the different treatments being significantly different ($P < .05$) when tested in a 2×3 contingency table. While there is no doubt that a larger number of treatments of a female produced more mutations, the data are not extensive enough to estimate the rate of increase in mutations with dose. The fact that the increase in lethal frequency with increased number of treatments was not statistically significant while the increase for translocations was, even though the latter involved fewer tests, suggests that the number of multiple events which produce translocations comprise a larger proportion of the total frequency of translocations than do the multiple-event lethals in the total lethal rate. More data are needed to accurately determine the rate at which translocations and lethals increase with triazine dose; these should be obtained following treatment of mature sperm (HERSKOWITZ 1955a) which are sampled for mutations at the same periods after treatment for all doses (see discussion which follows). It would appear to be equally efficient mutagenically to give two or three treatments of a 0.2% triazine solution spaced over a number of days as it is to give one treatment with a 1.0% solution.

The studies with triazine completely confirm SCHALET's (1955) finding with a nitrogen mustard that sperm whose utilization is delayed following treatment via vaginal douches possess a much larger number of translocations than those used soon after the treatment procedure, whereas there is no clear tendency in this direction

detectable for chromosome losses or recessive lethals (tables 2, 3). Although, in table 3, there is a significant increase in the lethals in brood 3 as compared with broods 1 + 2 in the single treatments with 0.2% triazine, for undetermined reasons, there is no evidence such as this for the six other cases in this table where it is possible to compare lethal frequency in a later brood with that in an earlier one.

To observe more easily the change in translocation frequency in successive broods, one can eliminate certain variations in treatments within and between experiments by dividing the translocation rate by the lethal rate for the same brood. When this was done (table 3), it can be seen that while the translocations ranged from 0.19 to 0.35 times as frequent as lethals in the different broods following the weakest treatment (single 0.2% triazine treatments), they increased from 0.19 to 0.64 times as frequent as lethals following two treatments with 0.2%, and went from 0.06 to 0.38 and from 0.38 to 0.88 times as frequent as lethals in going from broods 1 + 2 to brood 3 and from brood 3 to broods 4 + 5, respectively, following 1.0% treatment. In comparable experiments with mature sperm it was found (MULLER, HERSKOWITZ and OSTER unpublished) that a dose of X-rays giving 5.2% X-linked recessive lethals produced about 6.9% translocations of the types studied here (e.g. the translocations were 1.33 times as frequent as the lethals). It has been possible, then, under the conditions specified, to obtain an increase in the frequency of triazine-induced translocations from one-tenth (or less) of what one would get for a comparable frequency of lethals with X-rays to a frequency which is sensibly the same. No difference was noted between the relative numbers of different types of translocations in the X-ray work mentioned and those induced by triazine.

The results in table 1 show that, for equal concentrations, triazine is less toxic to the treated females than was a nitrogen mustard (table 7 in HERSKOWITZ 1955a). Triazine does so little harm to the treated females that even after the most severe treatments, with 1.0% solution and with 0.2% administered three times, 70% of the treated females produced adult offspring. The stability and inexpensiveness of triazine together with its equivalent mutagenicity (tables 2 and 3) as compared with nitrogen mustard (table 8 in HERSKOWITZ 1955a) make it a highly suitable chemical substance for investigative or classroom work with Drosophila.

SUMMARY

Postcopulatory vaginal douche treatment with triazine of mature sperm in females resulted in partial or complete losses of the sex chromosome, X-linked recessive lethal mutations, and translocations.

Additional treatments given several days apart produced more mutations. Two or three such douches with 0.2% solutions yielded approximately the same mutation rate as obtained from one treatment with 1.0% solution.

As was shown for a nitrogen mustard by SCHALET, the longer after treatment the sperm were held in the female, the higher was the translocation rate, so that for sperm held 9 or more days approximately as many translocations were produced by triazine as are produced by X-rays for the same lethal mutation rate.

ACKNOWLEDGMENT

It is a pleasure to thank MRS. BEVERLY POYNTER and also MRS. HERTA BURGER and MRS. BETSY BARDWELL for technical assistance in this investigation.

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CROSSING OVER AND NUCLEAR PASSING IN NEUROSPORA CRASSA[1]

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Received February 21, 1956

BY tetrad analysis in Neurospora 2-, 3-, and 4-strand double exchanges may be distinguished, and on a basis of random exchange are expected to occur in a ratio of 1:2:1. A significant departure from this ratio is indicative of chromatid interference. Previous investigations have indicated an excess of 2-strand double exchanges, or negative chromatid interference, across the centromere of the sex chromosome in Neurospora (LINDEGREN and LINDEGREN 1942).

Critical evaluation of chromatid interference between two immediately adjacent transcentromere regions is technically difficult, because apparent 2-strand double exchanges between these two regions may result from certain ascospore misarrangements. Even an infrequent occurrence of misarrangements might produce an apparent excess of 2-strand double exchanges across the centromere, because such double exchanges are themselves relatively rare events. LINDEGREN and LINDEGREN (1942) considered ascospore misarrangements an unlikely explanation of their nonrandom results.

The present paper reports data consistent with random exchange across the centromere of the Neurospora sex chromosome, as well as the occurrence of nuclear passing at the second meiotic division, detected by means of an independent centromere marker. Biochemical markers were used to increase the accuracy of scoring genotypes. A preliminary report has been published (HOWE 1954). STADLER (1956) has recently found additional evidence of random strand relationships during multiple crossing over in Neurospora and also reports meiotic nuclear passing. Less conclusive crossing over studies with genetically analyzed tetrads in other forms, some indicating randomness, some nonrandomness, have been reviewed by WHITEHOUSE (1942), PAPAZIAN (1952), and PERKINS (1955).

Cytological analyses of chromatid interference based upon relative frequencies of compensating and noncompensating chiasmata, or using structural heterozygotes, have suggested both nonrandomness (DARLINGTON and DARK 1932; HEARNE and HUSKINS 1935; DARLINGTON 1936; FRANKEL 1937; UPCOTT 1937; HUSKINS and NEWCOMBE 1941) and randomness (DARK 1936; GILES 1944). Genetic recombination values exceeding fifty percent, interpretable as an excess of 4-strand exchanges (positive chromatid interference), have been reported (CLAUSEN 1926; WELLENSIEK 1929; FISHER and MATHER 1936; WRIGHT 1947). Most of the Drosophila workers have concluded that 2-, 3-, and 4-strand double exchanges occur in random frequencies (ANDERSON 1925; BRIDGES and ANDERSON 1925; REDFIELD, 1930; MORGAN 1933; EMERSON and BEADLE 1933; BEADLE and EMERSON 1935; STURTEVANT and

[1] Taken from a dissertation offered in partial fulfillment of requirements for the degree of Doctor of Philosophy at the University of Wisconsin. Paper number 615 from the Department of Genetics.

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BEADLE 1936; WEINSTEIN 1936; WELSHONS 1955), but a significant departure from random expectations was reported by BONNIER and NORDENSKIÖLD (1937, see WELSHONS 1955 for critique).

MATERIALS AND METHODS

Three mutants of *Neurospora crassa* originally produced by BEADLE and TATUM (1945) were used. The mutant *ad-5(71104)* has a partial requirement for adenine (MITCHELL and HOULAHAN 1946), but reading of growth tests at 48 hours obviated scoring difficulty; *rib-1(51602l)* has a temperature-sensitive riboflavin requirement (MITCHELL and HOULAHAN 1946a); *vis(3717)* is a morphological mutant with no previously published description. Early growth (12–24 hours) on solid sucrose medium produces a circular patch with a regular margin easily distinguished from the irregularly spreading young hyphae characteristic of the wild allele. Fully developed growth on solid medium produces a limited mycelial patch with reduced aerial hyphae and no bright orange conidia. In liquid medium *vis(3717)* usually grows as a loose pellet at the bottom of the culture tube, producing neither pigment nor mycelial mat. The author is indebted to DR. R. W. BARRATT and to MRS. MARY B. MITCHELL for these mutant strains, and to DR. NORMAN H. GILES for wild types *73a-10a* and *74A-4B*.

In all, 1378 asci were dissected and classified, 38 (ascus numbers 701–738) from the cross *A ad-5* + ; *rib-1* × *a* + *vis*; + (table 1) and 1340 (ascus numbers 739–2078) from the cross *A ad-5 vis*; *rib-1* × wild type *73a-10a* (table 2). Tables 1 and 2 include only the 1199 asci in which at least one member of three or more different ascospore-pairs germinated and upon which tetrad analysis was based. When repre-

TABLE 1

Classification of 38 asci from the cross A ad +; r × a + v; + *in which at least one member of 3 or 4 ascospore-pairs germinated. Ascospore-pair arrangements are not necessarily ordered.* Rib-1 *(region IV) is not on the sex chromosome.* A, a*—mating type alleles,* ad*—adenine-5,* v*—visible;* r*—riboflavin-1*

Crossover classes	Regions	Ascospore-pair genotypes				Number of asci
Non		*A ad* + *r*	*A ad* + *r*	*a* + *v* +	*a* + *v* +	11
		A ad + +	*A ad* + +	*a* + *v r*	*a* + *v r*	15
	(IV)	*A ad* + *r*	*A ad* + +	*a* + *v r*	*a* + *v* +	1
Single	I	*A ad* + *r*	*a ad* + *r*	*A* + *v* +	*a* + *v* +	4
	I	*A ad* + +	*a ad* + +	*A* + *v r*	*a* + *v r*	3
Single	II	*A ad v r*	*a* + *v r*	*A ad* + +	*a* + + +	1
Single	III	*A ad v* +	*A ad* + +	*a* + *v r*	*a* + + *r*	1
	III	*A ad v r*	*A ad* + *r*	*a* + *v* +	*a* + + +	1
3-strand double	I–II	*A ad v r*	*a* + *v r*	*a ad* + +	*A* + + +	1
Total						38

TABLE 2

Classification of 1161 asci from the cross A ad v; r × a + +; + *in which at least one member of 3 or 4 ascospore-pairs germinated. Ascospore-pair arrangements are not necessarily ordered.* Rib-1 *(region IV) is not on the sex chromosome*

Crossover classes	Regions	Ascospore-pair genotypes				Number of asci
Non		*A ad v r*	*A ad v r*	*a + + +*	*a + + +*	407
		A ad v +	*A ad v +*	*a + + r*	*a + + r*	461
	(IV)	*A ad v r*	*A ad v +*	*a + + r*	*a + + +*	20
Single	I	*A ad v r*	*a ad v r*	*A + + +*	*a + + +*	39
	I	*A ad v +*	*a ad v +*	*A + + r*	*a + + r*	43
	I (& IV)	*A ad v r*	*a ad v +*	*A + + r*	*a + + +*	2
	I (& IV)	*A ad v r*	*a ad v +*	*A + + +*	*a + + r*	1
Single	II	*A ad v r*	*a + v r*	*A ad + +*	*a + + +*	20
	II	*A ad + r*	*a + + r*	*A ad v +*	*a + v +*	23
Single	III	*A ad v r*	*A ad + r*	*a + v +*	*a + + +*	66
	III	*A ad v +*	*A ad + +*	*a + v r*	*a + + r*	59
	III (& IV)	*A ad v r*	*A ad + +*	*a + + r*	*a + v +*	1
2-strand double	I–III	*A ad v r*	*a ad + r*	*A + v +*	*a + + +*	2
	I–III	*A ad v +*	*a ad + +*	*A + v r*	*a + + r*	1
	II–III	*A ad v r*	*a + + r*	*A ad v +*	*a + + +*	2
3-strand double	I–III	*A ad v +*	*a ad + +*	*a + v r*	*A + + r*	2
	I–III	*a ad v r*	*A ad + r*	*A + v +*	*a + + +*	2
	I–III	*a ad v +*	*A ad + +*	*A + v r*	*a + + r*	1
	I–III	*A ad v r*	*a ad + r*	*a + v +*	*A + + +*	3
	II–III	*A ad v r*	*a + + r*	*A ad + +*	*a + v +*	2
4-strand double	I–II	*a ad v r*	*a + v r*	*A ad + +*	*A + + +*	1
	I–III	*a ad v +*	*A ad + +*	*a + v r*	*A + + r*	1
	II–III	*A ad + r*	*a + v r*	*A ad + +*	*a + v +*	2
Total						1161

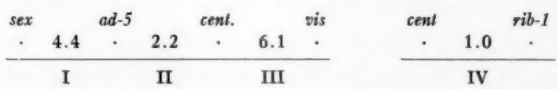
sentatives of fewer than three different ascospore-pairs in an ascus germinate, not all crossover types have an equal probability of being inferred, and hence 178 asci were omitted to avoid possible bias from germination deficiency. The remaining ascus, a 4-strand double exchange in a single region, was omitted because the corresponding 2- and 3-strand double exchanges could not have been detected. Table 3 shows the linkage relations of *sex*, *ad-5*, and *vis* on the sex chromosome (linkage group I) and of *rib-1* in linkage group VI. For convenience, the region between *rib-1* and its centromere is designated as region IV.

Crosses were made by simultaneous inoculations of parental strains onto fresh slants of supplemented WESTERGAARD-MITCHELL (1947) medium, followed by incubation in the dark at 25°C. Only asci containing eight uniformly dark, seemingly mature ascospores were dissected, but every ascus dissected was classified to the

TABLE 3

Classification of 1199 asci summarized from tables 1 and 2. Expected numbers computed on assumption of no interference

Crossover classes	Regions	Number of asci		Crossovers per region			
		Observed	Expected	I	II	III	IV
Non		915	918.2				21
Single	I	92	89.1	92			3
	II	44	41.6		44		
	III	128	127.3			128	1
Double	I & II	2	4.1	2	2		
	I & III	12	12.3	12		12	
	II & III	6	5.8		6	6	
Triple	I & II & III	0	0.6				
Total		1199	1199	106	52	146	25
C.o. freq.				.088	.043	.122	.021



maximum that ascospore germination would permit. All dissections were ordered. Ascospore isolates were heat-shocked immediately after dissection and then held at least two weeks to permit scoring of slow germinating types. Growth tests were run in duplicate on one member of each ascospore-pair, but on all members of asci having detectable ascospore misarrangements.

Equational division of centromeres at meiosis I, passing of the two center nuclei around each other at meiosis II, or dissecting errors could all produce ascospore misarrangements interpretable as 2-strand double exchanges between regions II and III, which bound the sex chromosome centromere. Thus an excess of apparent 2-strand double exchanges between two adjacent transcentromere regions may be a consequence of ascospore misarrangements rather than of actual negative chromatid interference. However, the strands involved in double exchanges between pairs of regions not adjacent to the centromere may be inferred from marker gene recombinations independently of ascospore order (LINDEGREN and LINDEGREN 1942).

An independent centromere marker was employed to detect asci resulting from meiotic nuclear passing or dissection errors, thereby making it possible to distinguish between false and legitimate 2-strand double exchanges in regions II and III. The independent centromere marker, *rib-1*, segregates at the first division in about 98 percent of asci. Passing, however, would convert a *rib-1* first division segregation into an apparent asymmetrical second division segregation, and similarly for *ad-5* and *vis* on the sex chromosome. Therefore, an ascus showing asymmetrical second division segregation arrangements of *ad-5*, *vis*, and *rib-1* would almost certainly

have been produced by meiotic nuclear passing or dissection error rather than by crossing over. An ascus produced by legitimate 2-strand double exchange in regions II and III would in all likelihood show first division segregation for *rib-1*, if independence of crossing over between tetrads is assumed.

Ascospore misarrangements resulting from centromere misassortment were not detectable as such, but failure to find negative chromatid interference makes it unlikely that appreciable misassortment of the sex chromosome centromere occurred. Dissecting errors could produce any ascospore misarrangements, but adjacent ascospores would most often be interchanged, and the correct order was usually inferable if enough ascospores germinated.

EXPERIMENTAL RESULTS

Chromosome interference

Table 3 summarizes the data from tables 1 and 2 on observed and expected crossovers in the three regions of the sex chromosome. The numbers of double crossovers by regions are too small for any definite conclusions, but these data best agree with an interpretation of no chromosome interference across the centromere (regions I and III and regions II and III), and positive interference in the left arm (regions I and II). No triple crossovers were found.

Chromatid interference

The ratio of 2-, 3-, and 4-strand double exchanges initially classified was 9:11:4. One of these 2-strand double crossover asci, 712, apparently involved regions II and III, directly across the centromere, and also showed apparent asymmetrical second division segregation of *rib-1*, the independent centromere marker (table 6). Ascus 712 almost certainly resulted from meiotic nuclear passing and is reclassified as a noncrossover ascus in the tables. The alternative interpretation requires a double crossover on the sex chromosome and a simultaneous crossover in the one map unit region between *rib-1* and its centromere. Similarly, Asci 1074, 1586, and 2022 are interpreted as nuclear passing or as a dissection error resulting in interchange of spores 3 and 6. This correction changes the double crossover ratio from 9:11:4 to 5:11:4 (table 4). This ratio does not differ significantly from the 1:2:1 expectation with random exchange between chromatids. Ordered ascospore arrangements of all double crossover asci are given in table 5.

TABLE 4

Observed and expected numbers of 2-, 3-, and 4-strand double crossovers among 1199 asci. Expected numbers computed on the assumption of no chromatid interference

Crossover class double	Regions			Totals	
	I-II	I-III	II-III	Observed	Expected
2-strand	0	3	2	5	5.0
3-strand	1	8	2	11	10.0
4-strand	1	1	2	4	5.0

TABLE 5

Ascospore arrangements in all double crossover asci. Ascospore number 1 is at the distal end of the ascus. Ascospores in parenthesis did not germinate but were inferable. Asci 701–738 are from the cross A ad +; r × a + v; +. *Asci 739–2078 are from the cross* A ad v; r × a + +; +

	Ascus number	Ordered ascospore genotypes							
		1	2	3	4	5	6	7	8
Two-strand									
I–III	1403	*a ad + r*	*a ad + r*	*A ad v r*	*A ad v r*	*A + v +*	*A + v +*	*a + + +*	*a + + +*
I–III	1666	*a ad + r*	*a ad + r*	*A ad v r*	*A ad v r*	*A + v +*	*A + v +*	*a + + +*	*a + + +*
I–III	1781	*A ad v +*	*A ad v +*	*a ad + +*	*a ad + +*	*A + v r*	*A + v r*	*a + + r*	*a + + r*
II–III	1306	*a + + r*	*a + + r*	*A ad v r*	*A ad v r*	*A ad v +*	*A ad v +*	*a + + +*	*a + + +*
II–III	2015	(*a + + r*)	*a + + r*	*A ad v r*	*A ad v r*	*a + + +*	*a + + +*	*A ad v +*	*A ad v +*
Three-strand									
I–II	717	*a + v r*	*a + v r*	*A ad v r*	*A ad v r*	*a ad + +*	*a ad + +*	*A + + +*	*A + + +*
I–III	850	(*a + v r*)	*a + v r*	(*A + + r*)	*A + + r*	(*a ad + +*)	(*a ad + +*)	*A ad v +*	*A ad v +*
I–III	977	*A ad + r*	*A ad + r*	*a ad v r*	*a ad v r*	*a + + +*	*a + + +*	*A + v +*	*A + v +*
I–III	1030	*a + v +*	*a + v +*	*A + + +*	*A + + +*	*a ad + r*	*a ad + r*	*A ad v r*	*A ad v r*
I–III	1069	*A ad v +*	*A ad v +*	*a ad + +*	*a ad + +*	*a + v r*	*a + v r*	*A + + r*	*A + + r*
I–III	1304	*a + + r*	*a + + r*	*A + v r*	*A + v r*	*a ad v +*	*a ad v +*	*A ad + +*	*A ad + +*
I–III	1323	*a ad + r*	*a ad + r*	(*A ad v r*)	(*A ad v r*)	*a + v +*	*a + v +*	*A + + +*	*A + + +*
I–III	1893	*A + v +*	*A + v +*	*a + + +*	*a + + +*	*A ad + r*	*A ad + r*	*a ad v r*	*a ad v r*
I–III	2040	*A + + +*	*A + + +*	*a + v +*	*a + v +*	*A ad v r*	*A ad v r*	(*a ad + r*)	(*a ad + r*)
II–III	882	*a + v +*	*a + v +*	*A ad + +*	*A ad + +*	*a + + r*	*a + + r*	*A ad v r*	*A ad v r*
II–III	1564	*a + v +*	*a + v +*	*A ad + +*	*A ad + +*	*A ad v r*	*A ad v r*	*a + + r*	*a + + r*
Four-strand									
I–II	1462	*A + + +*	*A + + +*	*A ad + +*	*A ad + +*	*a + v r*	*a + v r*	*a ad v r*	*a ad v r*
I–III	*1077	*a ad v +*	(*a ad v +*)	*A ad + +*	*A + + r*	*A ad + +*	*A + + r*	*a + v r*	*a + v r*
II–III	813	*A ad + r*	*A ad + r*	*a + v r*	*a + v r*	*A ad + +*	*A ad + +*	*a + v +*	*a + v +*
II–III	1420	*a + v +*	*a + v +*	*A ad + +*	*A ad + +*	*a + v r*	*a + v r*	*A ad + r*	*A ad + r*

* Also listed in Table 7.

Ascospore misarrangements

Two asci showed evidence of having been produced by meiotic nuclear passing rather than by crossing over (table 6). Ascus 712 showed apparent double crossovers in regions II and III, as well as apparent asymmetrical second division segregation of *rib-1*. Passing in ascus 2075 was detected by exceptional behavior of a *vis* modifier (see below). *Rib-1* also showed apparent asymmetrical second division segregation in ascus 2075, an indication of the reliability of the independent centromere marker technique. Asci 1074, 1586, and 2022 involve positional interchange of spores 3 and 6. This may be due to incomplete meiotic nuclear passing, meiotic and mitotic nuclear passing, or dissection errors. Similar explanations can account for 1001, 1913, and 2020 which show interchange of spores 4 and 6.

Fifty-five asci showed evidence of having resulted either from dissecting errors or from (third division) nuclear passing (table 7). These two causes are indistinguishable in any given ascus. Comparison of the frequency of misarrangements between

TABLE 6

Summary of asci showing evidence of nuclear passing or dissection errors. Asci 712, 1074, 1586, and 2022 might be regarded as 2-strand double exchanges except for post reduction of the riboflavin centromere marker. Ascopores in parentheses did not germinate but were inferable. Ascus 712 is from the cross A ad +; r × a + v; +; *the others are from* A ad v; r × a + +; +

Ascus number	Ordered ascospore genotypes							
	1	2	3	4	5	6	7	8
712	*A ad* + +	*A ad* + +	*a* + *v r*	*a* + *v r*	*A ad* + +	*A ad* + +	*a* + *v r*	*a* + *v r*
2075	*a* + + +	*a* + + +	(*A ad* + *r*)	*A ad* + *r*	*a* + *v* +	*a* + *v* +	(*A ad v r*)	*A ad v r*
1074	*A ad v r*	*A ad v r*	*a* + + +	*A ad v r*	*a* + + +	*A ad v r*	*a* + + +	*a* + + +
1586	*a* + + +	*a* + + +	*A ad v r*	*a* + + +	*A ad v r*	*a* + + +	*A ad v r*	*A ad v r*
2022	*a* + + *r*	*a* + + *r*	*A ad v* +	(*a* + + *r*)	*A ad v* +	(*a* + + *r*)	*A ad v* +	*A ad v* +
1001	*a* + + *r*	*a* + + +	*a* + + +	*A ad v r*	*A ad v r*	*a* + + +	*A ad v r*	*A ad v r*
1913	*A ad v r*	*A ad v r*	*A ad v r*	*a* + + +	*a* + + +	*A ad v r*	*a* + + +	*a* + + +
2020	*A ad v r*	*A ad v r*	*A ad v r*	*a* + + +	*a* + + +	(*A ad v r*)	*a* + + +	*a* + + +

TABLE 7

Summary of asci showing evidence of dissecting errors or third division nuclear passing among 1199 analyzed asci

Type of error. Transposition of ascospores	Number of asci	Number of asci per crossover class						
		NCO	NCO (IV)	SI	SII	SIII	SIII (IV)	DI-III
2 & 3	10		3	3	1	3		
4 & 5	32	24	2		2	3		1
3 & 6	3	3						
4 & 6	3	3						
6 & 7	3					2	1	
2 & 3; 4 & 5	2					2		
2 & 3; 6 & 7	1			1				
4 & 5; 6 & 7	1				1			
Total	55	30	5	4	4	10	1	1

ascospores two and three and between ascospores six and seven shows that misarrangements were about three times as frequent at the distal end of the ascus as at the proximal end, perhaps because of a greater tightness at the attached proximal end.

The preponderance of misarrangements between ascospores four and five (table 7) is at least partially due to the fact that four and five are the only two adjacent ascospores between which misarrangements can be detected in the most frequent ascus type, the noncrossovers. By assuming that the 32 interchanges between ascospores four and five represent about the average frequency of dissecting errors or third division passing, the total number of adjacent interchanges can be estimated, since practically all four-five interchanges are detectable.

If all seven possible adjacent ascospore interchanges had 32 misarrangements, the total number of asci with adjacent interchanges would be $32 \times 7 = 224$, or about 19 percent of the 1199 asci analyzed. Four of the seven possible adjacent interchanges are between sister ascospores and would not be detected in analysis. The correct order of detectable interchanges was usually inferable from the remaining members of the ascus.

To improve germination older asci were dissected, and this might have favored dissecting errors to some extent. Nevertheless, misarrangements did not increase appreciably with the period between crossing and dissecting, which averaged 35 days for all asci and 38 days for the 55 asci in table 7.

Modifiers of vis

Two additional markers were found in the course of this investigation, both modifiers of *vis* and linked to it. The first modifier, m_1, produces a browning of the agar and, about two thirds of the time, a few pale pink conidia; the complementary

TABLE 8

Frequency of crossovers between vis *and two* vis *modifiers,* m_1 *and* m_2, *in all asci in which these modifiers were determinable*

m_1		m_2	
Regions	Number of crossover asci	Regions	Number of crossover asci
a	96	c	108
b	50	d	197
a–b	1	c–d	20
Total asci	1200	Total Asci	1061

Map Distance, region a = $97/1200 \times 50 = 4.0$
Map Distance, region b = $51/1200 \times 50 = 2.1$
Map Distance, region d = $217/1061 \times 50 = 10.2$

sex · 4.4 · *ad-5* · 2.2 · *cent.* · 4.0 · m_1 · 2.1 · *vis* · 10.2 · m_2

a b d

c

crossover type produces a rapidly growing, fluffy mycelium which lacks conidia. The second modifier, m_2, produces a slowly growing, button-like mycelium; the complementary crossover type cannot be recognized. Table 8 gives the crossover data for these two modifiers and shows their locations.

The centromere-m_1 and the m_1-*vis* regions were not used as separate regions in the crossing over study, because m_1 decreased somewhat the accuracy of identifying progeny. The *vis*-m_2 region was not used, because the crossover complement of m_2 could not be recognized. The mutant m_1 was used to detect meiotic nuclear passing in one instance, however, in the following manner. This modifier always appeared coincidentally with crossing over in region III, locating it proximal to *vis*. In ascus 2075, however, m_1 appeared coincidentally with apparent crossovers in regions II and IV. The apparent second division segregation of *rib-1* was asymmetrical, as would be expected with passing. Ascus 2075 was therefore reclassified as a single crossover in region III (table 6).

Selection among the progeny

In 1378 total asci dissected, ascospore germination was 85.4 percent (table 9). Highest ascospore germination percentages were obtained with asci dissected six to seven weeks subsequent to setting up crosses. Table 9 shows germination according to ascospore position. Although suggestive of somewhat lower germination at the proximal end, the differences are not statistically significant.

Table 10 shows that neither of the two alleles at the *sex*, *ad-5*, or *vis* loci had a significant selective advantage. However, the wild type allele at the *rib-1* locus survived more often than did the mutant allele, and the difference is highly significant. Because *rib-1* was used only as an independent centromere marker, whereas crossing over was studied in the sex chromosome, this differential effect of *rib-1* could not influence the crossing over analysis made. The absence of any significant effect of the sex chromosome markers on ascospore germination, together with the

TABLE 9

The relation between ascospore germination and position of the ascospore in all 1378 asci dissected. Position 1 is the most distal

Ascospore position	Number of ascospores		Percent germination
	Germinated	Non-germinated	
1	1176	202	85.3
2	1194	184	86.6
3	1194	184	86.6
4	1184	194	85.9
5	1182	196	85.8
6	1183	195	85.8
7	1156	222	83.9
8	1142	236	82.9
Total	9411	1613	85.4

$\chi^2_7 = 13.7$, $P = 0.07$.

TABLE 10

Effect of marker genes on ascospore germination in all 1378 asci dissected. The number of germinated and non-germinated, but inferable, ascospores is given for each pair of alleles. The chi-square is the interaction chi-square computed for each set of four

Alleles	Number of ascospores		x_1^2	Prob.
	Germinated	Non-germinated, genotype inferable		
A	4736	403	1.62	>.05
a	4675	436		
ad-5	4728	412	.40	>.05
ad-5$^+$	4683	427		
vis	4727	419	.02	>.05
vis$^+$	4684	420		
rib-1	4632	496	30.2	<.001
rib-1$^+$	4779	343		

high overall germination rate, makes it extremely unlikely that germination deficiency could have appreciably biased the estimates of crossing over and interference.

Spindle orientation

Table 11 gives first meiotic division spindle orientations for all asci for which these could be inferred. There was no evidence for preferential segregation in either chromosome, nor for any interaction between the *sex* and *rib-1* tetrads. Second meiotic division spindle orientation data also indicate randomness, since the frequencies of symmetrical and asymmetrical arrangements are not significantly different (table 12).

TABLE 11

First meiotic division spindle orientation in the 1188 asci for which spindle orientation was inferable

Tetrads	Ordered ascospore-pair arrangements				Number of asci	Totals	x_1^2	Prob.
	1.2	3.4	5.6	7.8				
sex	*A ad v r*	*A ad v r*	*a + + +*	*a + + +*	298	620	2.28	>.05
	A ad v +	*A ad v +*	*a + + r*	*a + + r*	322			
	a + + +	*a + + +*	*A ad v r*	*A ad v r*	266	568		
	a + + r	*a + + r*	*A ad v +*	*A ad v +*	302			
rib-1	*A ad v r*	*A ad v r*	*a + + +*	*a + + +*	298	600	.12	>.05
	a + + r	*a + + r*	*A ad v +*	*A ad v +*	302			
	a + + +	*a + + +*	*A ad v r*	*A ad v r*	266	588		
	A ad v +	*A ad v +*	*a + + r*	*a + + r*	322			
Tetrad Interaction	*A ad v r*	*A ad v r*	*a + + +*	*a + + +*	298	564	3.03	>.05
	a + + +	*a + + +*	*A ad v r*	*A ad v r*	266			
	A ad v +	*A ad v +*	*a + + r*	*a + + r*	322	624		
	a + + r	*a + + r*	*A ad v +*	*A ad v +*	302			

TABLE 12

Second division segregations for four loci in all asci from tables 1 and 2

Segregation type	Locus				Total		x_1^2	Prob.
	sex	*ad-5*	*vis*	*rib-1*	Obs.	Exp.		
Symmetrical	72	27	68	12	179	189	1.06	>.05
Asymmetrical	83	25	78	13	199	189		

DISCUSSION

The conclusion has been reached that crossing over was at random between chromatids along that portion of the sex chromosome analyzed. Meiotic nuclear passing seems to occur, but with very low frequency.

LINDEGREN and LINDEGREN (1942) analyzed four regions of the Neurospora sex chromosome delimited by the *sex* locus and three morphological markers, as follows: region I, *sex-gap*, 4 units; region II, *gap*-centromere, 3.5 units; region III, centromere-*crisp*, 3.5 units; region IV, *crisp-pale*, 8 units. Departures from random exchange between chromatids were found.

In the present investigation only about 0.2–0.5 percent of the asci analyzed showed evidence of meiotic nuclear passing. Alternatively, these may be due to dissection errors or postmeiotic transpositions. MCCLINTOCK's (1945) analysis of arrangements of visibly different ascospores in 869 asci revealed seven arrangements (0.8 percent) that could be attributed to passing. Table 2 (LINDEGREN and LINDEGREN 1942) gives ordered arrangements of triple and quadruple crossovers found. Four of the nine triple crossovers, 3243, 2968, 3150, and 3249, become single crossovers on an assumption of passing, and all four involve exchanges directly across the centromere, with asymmetrical *gap* and *crisp* arrangements. On the basis of this reinterpretation, about 0.3 percent passing occurred in the LINDEGRENS' study, and the frequency might have been somewhat higher, if all occurrence of passing had been discernible among their double crossovers.

The frequency of passing may tend to vary in different stocks, but evidence so far accumulated indicates that frequencies somewhat under one percent are to be expected. In studies depending upon ascospore order, two independent centromere markers instead of one would be more precise in validating critical asci, but would require more time and labor in classifying progeny.

The ratio of 2-, 3-, and 4-strand double exchanges reported by LINDEGREN and LINDEGREN (1942) was 28:13:15, a significant departure from random expectation ($P < .01$). The LINDEGRENS' double crossover ratio for their first three regions, 13:4:3, also differed significantly from expectation ($P < .05$), and from the writer's double crossover ratio, 5:11:4, for three similarly placed regions ($P < .05$). The finding of evidence for nuclear passage, undetected except by a centromere marker, suggests that at least some of the LINDEGRENS' excess of 2-strand doubles might be attributed to this cause. In fact, the original classification ratio of 9:11:4 before correction for nuclear passage or errors of dissection does not differ significantly from LINDEGREN's 13:4:3.

PERKINS (1955) showed that evidence for chromatid interference is greatly reduced in the total data if retabulation is made on the assumption that all the LINDEGRENS' 2-strand double crossovers in regions II and III, about one percent of asci, resulted from various misarrangements. The LINDEGRENS' 15:7:6 double crossover ratio for regions I and IV only, however, shows evidence of negative chromatid interference not readily attributable to misarrangements, since these two regions are not adjacent to the centromere.

LINDEGREN and LINDEGREN (1942) reported an excess of triple and quadruple exchanges. Their total multiple exchanges were not in excess, however, because the most frequent multiple type, the doubles, were fewer than expected (total multiples observed:total multiples expected = 70:80.06 = 0.87). Moreover, reinterpretation of the ordered arrangements of their nine triple crossovers, as discussed above, indicates that four of the triples probably resulted from passing and should be reclassified as single crossovers. The expected number of triples was 4.8. Their quadruple exchanges, however, are in marked excess even if reinterpreted on the passing hypothesis, but not if assumptions of nonadjacent rearrangements, as by centromere misassortment, are made (see PERKINS 1955).

SUMMARY

Crossing over was studied in the sex chromosome of *Neurospora crassa* by means of genetically analyzed tetrads. Data were obtained from 1199 asci having at least one member of three different ascospore-pairs germinating. Three regions on the sex chromosome were considered, regions I and II to the left of the centromere and region III to the right.

The ratio of 2-, 3-, and 4-strand double crossovers found was 5:11:4 and does not differ significantly from the 1:2:1 chance expectation. Thus there is no suggestion in our data for chromatid interference, or of any interference across the centromere.

An independent centromere marker technique was employed for detecting meiotic nuclear passing or errors in dissection. Such misarrangements were of rare occurrence, having been found in but 0.2–0.5 percent of the asci analyzed. The effects of meiotic nuclear passing and other misarrangements upon conclusions drawn from chromatid interference data are considered.

Differences between the present findings and those of LINDEGREN and LINDEGREN (1942) are discussed.

ACKNOWLEDGMENTS

The writer is deeply indebted to DR. JAMES F. CROW and to DR. JOSHUA LEDERBERG for much helpful advice and criticism throughout the course of this investigation and to DR. CROW for help in the writing of the manuscript. He is also indebted to DR. DONALD M. BOONE for generous help with techniques.

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DOUBLE CROSSING OVER IN NEUROSPORA

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Received February 21, 1956

A CROSSOVER involves exchange of genetic material between only two of the four products of meiosis. We should like to know whether there are any special properties of these two strands of the tetrad which enable them to participate in crossing over. Such knowledge might help us understand the mechanics of the crossing over process and would perhaps shed some light on the physical nature of the chromosomes themselves. One approach to this problem is the study of strand relationships in double crossovers (two crossovers in the same tetrad). This type of study can only be made in a situation in which at least two of the products of meiosis can be recovered and analysed genetically. Analysis of attached-X females in Drosophila permits a comparison of the frequencies of 2-strand and 4-strand double crossovers (BEADLE and EMERSON 1935; BONNIER and NORDENSKIÖLD 1937; WELSHONS 1955).

Tetrad analysis in Neurospora permits a direct scoring of 2-strand, 3-strand, and 4-strand double crossovers. With random association of non-sister strands, we would expect these three types of double crossover to occur with a frequency ratio of 1:2:1. Any significant deviation from this ratio is considered to be a demonstration of "chromatid interference". LINDEGREN and LINDEGREN (1939, 1942) studied double crossing over in neighboring regions of the same chromosome arm of Neurospora, and they found no evidence of chromatid interference. However, the same authors (LINDEGREN and LINDEGREN 1937, 1942) reported that among double crossovers involving regions in opposite chromosome arms, the majority involved only two strands. Recent work has not confirmed this report of an excess of 2-strand double crossovers. HOWE (1956), in a study of the same linkage group as was used by LINDEGREN and LINDEGREN, found no evidence of chromatid interference across the centromere. The findings of the present report, involving double crossing over across the centromere of another linkage group of *Neurospora crassa*, are in complete agreement with those of HOWE.

METHODS

All crosses in this study were segregating for the ascospore mutant, *asco* (37402). Spores carrying this mutant gene remain colorless while the wild type spores turn black. Crossing over in the region between the *asco* locus and the centromere can be detected in the whole ascus by the patterns of 2nd division segregation for the ascospore character (STADLER 1956). Only these asci need be dissected and grown to test for other segregating mutants. This method of selection of crossover asci greatly reduces the labor involved in a study of chromatid interference. The experiments reported below involve the analysis of 8455 asci for double crossing over, but the time-consuming operations of dissection and growth tests were only performed on the 1622 asci with patterns of 2nd division segregation for *asco*.

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One disadvantage of the mutant *asco* is that it is nearly always lethal. Only a small fraction of those spores carrying it will ever mature and grow. This means that only the spores carrying the wild allele of *asco* can be grown and classified for other segregating markers. However, even this amount of information is sufficient to determine the strand relationships of double crossovers in certain situations.

Comparative frequencies of 2-strand and 4-strand double crossovers

In these experiments, *asco* was crossed to a strain carrying a linked mutant in the opposite chromosome arm. Asci with crossing over between the *asco* locus and the centromere (region I) were selected by their patterns of 2nd division segregation. The mature (*asco*$^+$) spore pairs from these asci were grown and tested for the presence of the other segregating mutant. If we assume that both of the marked regions are short enough to insure that there will not be more than a single crossover within a region, the following interpretation can be made (see fig. 1): Those asci with no crossover between the centromere and the linked mutant (region II) have a single recombination event, resulting in one mutant and one wild spore pair. A crossover in region II making a 4-strand relationship with the one in region I results in double recombination, and both spore pairs are wild. A 2-strand double crossover across the centromere results in no recombination of the marker genes, and both spore pairs are mutant.

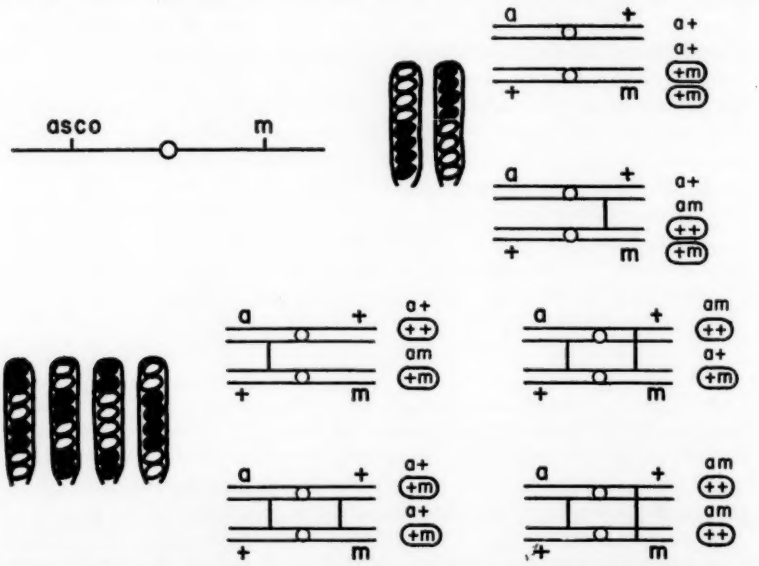

FIGURE 1.—Spore patterns and ascus types in a cross of *asco* to a linked mutant in the opposite chromosome arm. Ovals are drawn around the genotypes of those spore pairs which carry the wild allele of *asco* and can be grown and tested for the other segregating mutant. Above: asci with no crossing over between the *asco* locus and the centromere. Below: asci with crossing over between *asco* and the centromere.

TABLE 1

Counts of 2-strand and 4-strand double crossovers

Cross number	Mutant crossed to *asco*	Asci with 2nd division segregation for *asco*		
		Single recombination	Non-recombination (2-strand double crossover)	Double recombination (4-strand double crossover)
1	*rib-1*	395	1	1
2	"	13	0	0
3	"	2	0	0
4	"	19	1	1
5	"	19	0	0
6	"	14	0	0
7	"	12	0	1
8	"	242	1	2
9	"	10	1	1
10	"	8	0	0
11	"	10	0	1
12	"	7	0	1
13	"	33	0	0
14	"	65	3	1
15	"	17	0	0
16	"	3	1	0
17	"	602	5	3
Total	"	1471	13	12
18	*tryp-2*	105	12	9

A 3-strand double crossover yields one mutant and one wild spore pair and is thus indistinguishable from the single crossover ascus.

The results of these experiments are shown in table 1. It can be seen that there is no significant deviation from the 1:1 ratio of 2-strand and 4-strand double crossovers expected in the absence of chromatid interference. In cross # 1 there were three more asci with patterns of 2nd division segregation for *asco* and with both spore pairs mutant at the *rib-1* (51602) locus. These were not listed in the table with the 2-strand double crossovers, because they were shown by a method similar to that of HOWE (1956) to have resulted almost certainly from meiotic nuclear passing rather than crossing over. Segregating markers located near the centromeres of two other linkage groups (5801 in group III and mating type in group I) showed coincident patterns of 2nd division segregation with *asco* and *rib-1* in these asci. A less precise test for meiotic nuclear passing was made in all other crosses to *rib-1* (# 2 through 17), involving a marked locus in only one other linkage group. This was the mating type locus, which is about 6 units from the centromere. The test revealed that two of the twelve asci classified as 2-strand double crossovers in these crosses may have resulted instead from meiotic nuclear passing. In the cross to *tryp-2* (75001), no check was made for such aberrant meiotic movements. Crossing over was so frequent in region II of this cross that the results could only have been obscured by meiotic nuclear passing occurring much more often than has been observed in other crosses.

TABLE 2

Test for chromosome interference

Cross number	Mutant crossed to *asco*	2 & 4-strand double crossovers among asci with 2nd division segregation for *asco*	Recombination asci among those with 1st division segregation for *asco*	Crossover frequency in region II (map units)	
				With crossing over in region I	With no crossing over in region I
1	*rib-1*	2 in 397 asci	2 in 104 asci	0.50	0.96
8	*rib-1*	3 in 245 asci	3 in 100 asci	1.22	1.50
17	*rib-1*	8 in 610 asci	7 in 198 asci	1.31	1.77
18	*tryp-2*	21 in 126 asci	32 in 100 asci	16.7	16.0

In crosses 1, 8, 17 and 18, asci with no crossover in region I were analysed for crossing over in region II (table 2). These data enable us to test for the occurrence of chromosome interference (a correlation of crossing over in two neighboring regions). However, we must make an assumption as to the frequency of 3-strand double crossovers among the asci with crossing over in region I. In the calculations shown in table 2, it has been arbitrarily assumed that the observed 2-strand and 4-strand double crossovers represent one half of the total double crossovers: i.e., that there is no chromatid interference. The calculations indicate no chromosome interference.

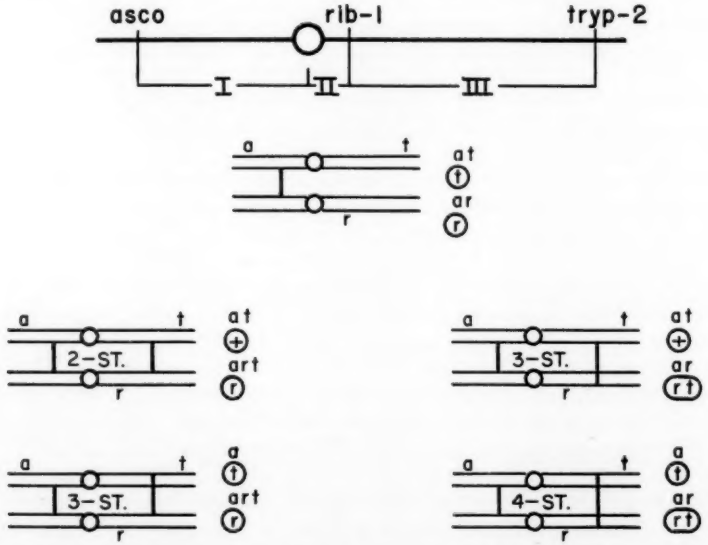

FIGURE 2.—Expected ascus types in the cross *ascotryp-2* × *rib-1* with crossing over between *asco* and the centromere (region I) and between *rib-1* and *tryp-2* (region III). Encircled genotypes are those of spore pairs carrying the wild allele of *asco* which can thus be tested for the other segregating mutants. Genotypes are abbreviated by listing only the mutants (and not the wild alleles) present.

Comparative frequencies of 2-strand, 3-strand and 4-strand double crossovers

The cross analysed in this experiment was segrating for *asco* and two linked mutants in the opposite chromosome arm: *rib-1* and *tryp-2*. The *rib-1* locus is very near the centromere. Crossing over was scored in three marked regions: *asco* to centromere (region I), centromere to *rib-1* (region II), and *rib-1* to *tryp-2* (region III). 2-strand and 4-strand double crossovers involving regions I and II could be analysed as in the previous experiments; these data are listed in table 1 as cross # 17. Among double crossovers between regions I and III, the 2-strand and 4-strand types and one of the two 3-strand types are all detectably different from each other and from other crossing over events of the same or lower rank (see fig. 2).

Counts of segregation patterns of 5085 asci from the cross *asco tryp-2* × *rib-1* revealed a frequency of 2nd division segregation for *asco* of 20.8% (4026 1st division segregations, 523 alternating and 536 symmetrical 2nd division segregations). 781 asci with patterns of 2nd division segregation were dissected, and the two mature spore pairs of each ascus were transferred to slants of complete medium. There were 610 asci in which both spore pairs were viable. These were tested for riboflavin and tryptophane requirements. The results of the analysis of these asci are shown in table 3.

TABLE 3

Analysis of 610 asci with 2nd division segregation for asco *from the cross* asco tryp-2 × rib-1

Number of asci	Genotypes of viable (*asco*$^+$) spore pairs	Simplest crossing over events to yield this type of ascus
452	*rib-1, tryp-2*	Single crossover in region I*
43	*rib-1*, +	2-strand double in regions I and III
54	*rib-1 tryp-2*, +	3-strand double in regions I and III
53	*rib-1 tryp-2, tryp-2*	4-strand double in regions I and III
4	*rib-1, rib-1*	2-strand double in regions I and II
3	*tryp-2, tryp-2*	4-strand double in regions I and II
1	*rib-1 tryp-2, rib-1*	triple crossover in regions I, II and III

* This class also includes the undetectable 3-strand double crossovers involving regions I and III.

TABLE 4

Analysis of 200 asci with 1st division segregation for asco *from the cross* asco tryp-2 × rib-1

Number of asci	Genotypes of viable (*asco*$^+$) spore pairs	Simplest crossing over events to yield this type of ascus
129	*rib-1, rib-1*	No crossover
62	*rib-1 tryp-2, rib-1*	single crossover in region III
4	*rib-1, tryp-2*	single in region II
2	*rib-1*, +	2-strand double in regions II and III
2	*tryp-2, tryp-2*	4-strand double *within* region I*
1	*rib-1 tryp-2, tryp-2*	3-strand double in regions II and III

Crossing over in region III in asci with no crossing over in region I: 65 exchanges in 198 asci—16.4 map units.

Crossing over in region III in asci with crossing over in region I (data from table 3): 201 exchanges in 610 asci—16.5 map units.

* Because these two asci had crossing over in region I, they were excluded from the above calculations testing for chromosome interference.

The observed ratio of 2-strand, detectable 3-strand, and 4-strand double crossovers of 43:54:53 does not depart significantly from the 1:1:1 ratio expected in the absence of chromatid interference.

A test for chromosome interference was also made on this cross. 200 asci with patterns of 1st division segregation for *asco* were grown and classified for *rib-1* and *tryp-2*. These data are shown in table 4. In comparing the frequencies of crossing over in region III with and without crossing over in region I, the assumption is made that the 150 double crossovers of the three detected types represent 75% of the total double crossovers between regions I and III (the one triple crossover ascus must be added to this total for the calculation). The calculation reveals that crossing over in region III is not correlated in any apparent way with that taking place in region I.

The frequencies of crossing over in regions I and III of this experiment are high enough to justify some concern over possible double crossovers *within* one of these regions. Such undetectable events might conceivably occur with a low frequency, so it is necessary to determine how they might obscure the results. By working out the expected ascus types for all combinations of double crossovers within one region coupled with single or noncrossovers in the other regions, it can be shown that the only such event which would bias the results in favor of one of the three types of detected double crossovers between regions I and III would be a single crossover in region I together with a 4-strand double crossover in region III. Regardless of the strand relationships of the crossover in region I with those in III, such an event would be erroneously scored as a detectable 3-strand double crossover between the two regions. 4-strand double crossovers within region III could not be detected in the analysis of the asci with 2nd division segregation for *asco*, but such an event would be detectable in an ascus with 1st division segregation for *asco*. It is reassuring to note that no such event occurred among the 200 asci in which it might have been detected.

The counts of double crossovers involving regions I and III are not affected by meiotic nuclear passing. Such an event would be scored as a 2-strand double crossover involving regions I and II. Five asci among the 610 analysed fell into this class (4 *rib-1*, *rib-1* and 1 *rib-1 tryp-2*, *rib-1*). Mating types were determined on these strains as a partial check for meiotic nuclear passing. In one of the five asci the mating type locus showed a coincident pattern of 2nd division segregation with all the other markers. This one ascus may have resulted from meiotic nuclear passing rather than crossing over.

Of the 781 asci with *asco* segregating in the 2nd division only those 610 in which

TABLE 5

Frequencies of genotypes among asco$^+$ *progeny of the cross* asco tryp-2 × rib-1

	rib-1	*tryp-2*	*rib-1 tryp-2*	+	Total
Cultures from the 610 viable 2nd division segregation asci	504	510	109	97	1220
Cultures from the 89 partially viable 2nd division segregation asci	46	33	6	4	89

Test for homogeneity: chi-square = 4.34 $P > 0.20$.

both spore pairs grew could be included in the experiment. It would be desirable to know whether the 171 asci which could not be included were a selected group in any sense. In 89 of these asci, one of the spore pairs was viable. These 89 cultures were classified for riboflavin and tryptophane requirements. In table 5 the frequencies of the four possible genotypes among these cultures are compared to the frequencies among the 1220 cultures of the asci counted in the experiment. There is no apparent selection against a particular genotype in the partially viable asci.

DISCUSSION

LINDEGREN and LINDEGREN (1937, 1942) reported strong negative chromatid interference in double crossovers involving both chromosome arms; 37 out of 64 involved only two strands. There has been considerable speculation as to whether some of the apparent 2-strand double crossovers observed by LINDEGREN and LINDEGREN might have resulted from meiotic nuclear passing (WHITEHOUSE 1942; PERKINS 1955; HOWE 1956). HOWE (1956) has devised a technique for detecting meiotic nuclear passing, thus ruling out this possible source of error. This method has also been used in the experiments reported here.

In designing an experiment to study double crossing over, some compromise must be made between precision and yield. HOWE's experiment involved short map regions and was thus precise in the sense that undetected double crossovers *within* regions were highly improbable. Because the regions were so short, he observed only a small number of double crossovers (20 in 1199 asci). The ratio of the 2, 3, and 4-strand types of 5:11:4* is certainly in good agreement with the 1:2:1 ratio expected with no chromatid interference. But because the numbers are small, the result is compatible with quite a wide range of ratios of the three types.

The present experiment has sacrificed some degree of precision in favor of yield of double crossovers. In the cross of *asco tryp-2* × *rib-1*, the 610 asci with 2nd division segregation for *asco* represented a total of 2928 asci and yielded 150 detectable double crossovers involving regions I and III. The relative frequencies of the 2, 3, and 4-strand types are again in good agreement with the expectation with no chromatid interference. In this case the numbers are large enough to put somewhat narrower limits on the statistical fluctuation of the ratio.

The present findings, like those reported by HOWE, indicate that crossing over events in one chromosome arm are independent of those going on in the other arm of the same tetrad. They tell us nothing about the relationship of crossovers in neighboring regions of the same chromosome arm. Studies in other forms have demonstrated that the interference relations between regions on opposite sides of the centromere are different from those between regions on the same side.

SUMMARY

Crossing over analyses involving 8455 asci have been made on marked regions on both sides of the centromere of linkage group VI of *Neurospora crassa*. Tests were

* Two of these involved regions in the same arm. The ratio for doubles across the centromere was actually 5:10:3.

made for chromatid and chromosome interference. The results indicate that crossing over events in one arm of a chromosome are independent of those in the other arm.

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